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Guidelines for Physicochemical Characterization of Biomaterials

Report of the National Heart, Lung, and Blood Institute Working Group

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GUIDELINES FOR
PHYSICOCHEMICAL CHARACTERIZATION OF BIOMATERIALS

Devices and Technology Branch
National Heart, Lung, and Blood Institute

April 1979

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CHAPTER I

BACKGROUND

In 1977, the National Heart, Lung, and Blood Institute undertook a review of its activities in biomaterials development with the aims of defining the needs for such activities in support of the Circulatory Assist and Artificial Heart Program, assessing the progress that had been made in meeting those needs, and establishing the appropriate directions and goals for future efforts. A Task Force on Biomaterials was appointed to advise the Institute on these matters. It met several times from February to July of 1977, obtained and considered the opinions of representatives of the scientific community, and reported its conclusions and recommendations to the Cardiology Advisory Committee in October 1977.

Its findings reaffirmed the need for materials research in support of the Circulatory Assist and Artificial Heart Program objectives, particularly in the development of materials of adequate durability and blood compatibility. The report pointed out that while the field of blood compatible materials had advanced enormously in a decade, there were still significant gaps in basic understanding which had to be addressed. Moreover, part of the difficulty arose from the wide variation in methods for testing and evaluation of materials and, in many instances, the unavailability of sufficiently reliable methods. It recommended that, rather than undertaking the development of specific new materials at this time, priority be given to the development of reliable approaches to the characterization and evaluation of materials and of blood-material interactions. These were viewed as necessary prerequisites to understanding the properties of materials exhibiting blood compatability and to designing materials that exhibited such properties. As part of this effort, the task force recommended that "...immediate priority... be given to the development of provisional standards for the physicochemical characterization of materials and for assessing blood compatibility." The task force proposed that these standardized protocols be evolved by one or more working groups comprising representatives from the appropriate scientific disciplines. The protocols were to be detailed and readily applicable in laboratories engaged in biomaterials development. The task force further proposed that reference materials should be designated to which the standardized protocols could first be applied.

Based on the biomaterials task force recommendation, the Working Group on Standards for the Physicochemical Characterization of Biomaterials for Cardiovascular Application and the Working Group on Standards for the Blood Compatibility of Biomaterials were formed and met initially in Philadelphia, Pennsylvania, on April 9, 1978. The membership of each of these groups is listed in the front of this report. The primary charge to the former working group was to establish the physicochemical characterization standards recommended by the task force where it could be determined that adequate techniques and protocols now exist and are agreed upon in the scientific community, and to identify "gap areas" where further research was necessary to establish methods for such characterization or to confirm the validity of proposed methods.

The working group on physicochemical characterization met several times in the performance of its assigned tasks, conducted open discussions with a representative segment of the scientific community, and solicited written comments relative to its deliberations. The report which follows is the result of those activities, and is respectfully submitted to the National Heart, Lung, and Blood Institute.

CHAPTER II

OVERVIEW

The characteristics required of a material in a cardiovascular application vary markedly with the particular application. An intra-aortic balloon pump to be used for a period of several days in a high blood flow environment has requirements of blood compatibility far different from those of a permanently implanted small caliber vascular prosthesis, and requirements of fatigue resistance far different from those of a bladder in a left ventricular assist device designed for 1 to 2 years of use. Moreover, even where the ultimate functional requirements of a material can be specified, the imperfect state of our understanding of blood-material interactions make it highly questionable that those functional requirements can be translated into values of physicochemical properties of the material. Therefore, the working group agreed that development of performance standards for materials would be inappropriate at this time. Instead, it set as its goal the development of a compendium of appropriate test methods for identifying and classifying materials properties.

This report outlines these test methods in an order described in the following paragraphs. The descriptions, however, are not meant to be comprehensive; therefore, many details and a good deal of the background necessary to carry out each of the procedures are not included. Since individuals using this report will come from widely different fields, it may not always be apparent to the user what information is lacking. For this reason, the reader should always consult the referenced texts and journal articles before attempting to use these test procedures.

The characterization of materials requires techniques of varying degrees of complexity and sensitivity. Sample sizes range from a scale of centimeters to a scale of Angstroms. Analyses can be directed at assessing a range of characteristics from elemental composition to subtle domain structure; from macroscopic yield stress or elasticity to chemical bond strength or polymer chain entanglement; from physical appearance to X-ray spectra. In addition, materials can be characterized in terms of their effects on biological systems, which often reflect differences in material not easily detectable by other methods.

The test procedures involved in each of these kinds of characterizations range from simple to complex, correlating generally with increases in cost, in sophistication of equipment required and in the expertise necessary for appropriate analysis and interpretation of results. As might be expected, however, the more sophisticated tests provide greater sensitivity to subtle material differences. If the parameters that determine blood-materials compatibility were known, it would be possible to limit the tests to those which are necessary to specify the relevant parameters.

Since we have not yet reached that state of understanding, the working group has included in the following sections all test methods for which there is a consensus that reproducible and meaningful data can be obtained which relate to an identifiable characteristic or characteristics of the material under test.

It is clear, however, that not all laboratories are equipped to perform each of these tests and that each test is not necessary for all purposes. Therefore, the test methods have been divided into categories referred to as level I and level II. The level I tests comprise those few that the working group considers minimally necessary to characterize or to assure the proper identification of a material to be used in a cardiovascular application. In all instances these are tests that can be performed with a minimum of effort and with relatively simple equipment. It appears reasonable and the working group recommends that performance of the applicable tests in this category be required in the course of materials development, and also in quality assurance for the raw materials, for the individual components of cardiovascular devices, and for the finished devices themselves.

The remaining test methods that the working group considers to be well-defined and meaningful are classified as level II tests. These are most appropriate for use in materials development and in conjunction with the procedures to test blood-material interaction. We envision that the use of these tests in materials development will be determined by an iterative process. That is, more sensitive characterization procedures would be employed only as less sensitive ones fail to distinguish the properties of materials that respond differently to the blood-material interaction tests or fail in use in distinguishable ways.

Methods that do not presently meet the requirements of requirements of reproducibility or interpretability have been identified as level III tests. In many instances, the potential exists for these methods to be developed further so that the precise relationship between the measured parameter and particular physical or chemical properties of the material can be clarified. Indeed, in certain instances they represent the state-of-the-art methods so new and sophisticated that only a very few laboratories are capable of using them and thus the practicality of their broad use is limited. The iterative approach discussed above should provide a rational basis for establishing priorities for the development of these level III physicochemical characterization techniques; that is, if none of the presently measurable parameters provide a satisfactory correlation with blood-material behavior, development of a new measurement techniques would have to be undertaken as as high priority of the program. A certain number of test procedures have been excluded entirely from the report. Generally, these are procedures that appear to have little potential for advancing our understanding of blood-material interactions either because they cannot be related in any obvious way to materials properties, they are highly sensitive to interfering artifacts, or they provide information more accurately or easily provided by other methods included here.

From a functional point of view, the properties to be measured include those related to identification of surface properties, mechanical strength and behavior, chemical and mechanical stability, toxicity, and sterilizability. These, then are the broad categories addressed in the characterization procedures discussed in the following sections. The properties of interest depend upon a great many factors: elemental composition; molecular (or monomeric) structure; polymeric or long-range structure; extent of heterogeneity; and the type and amount of minor components (e.g., unreacted monomers, catalyst, plasticizers, etc.). Each factor must be assessed by separate test procedures. Table 1 presents a matrix categorizing the test procedures to be described according to level and functional category.

The applicability of these procedures for materials characterization is a function of the nature of the material. In the course of the development of devices for cardiovascular application, a great many different kinds of material have been considered and are being used. These include synthetic polymers, (hydrophobic, hydrophilic, crystalline, amorphous, etc.), ceramics, metals, carbons, natural macromolecules, modified tissue, and unaltered cellular linings. In addition, the physical form of the material varies widely. Some are smooth, others are fabricated as velours, woven or porous materials. Some are composites, or are applied as coatings on other materials. While we recognize that not all of the listed procedures will be applicable to each material, we believe that many will be and that where limitations occur, they are more likely to arise due to the difficulty of preparing appropriate samples, a consequence of physical structure rather than chemical structure. Therefore, no explicit consideration has been given to these differences in specifying the test protocols. It is likely that the procedures will be inappropriate for characterizing materials which depend upon cellular linings for their blood compatibility and it is possible that they will be of limited usefulness in characterizing the surfaces of nonsmooth materials.

Material properties are affected by conditions of synthesis or polymerization and are altered by fabrication steps, packaging, sterilization, shipping, storage, and subsequent environmental and temporal stress factors. Thus, the properties are important and continually evolving functions of the material's history. As a consequence, great care must be taken to record the history of any material in terms of each of these variables, to specify the form in which the material is tested, and to repeat many aspects of its characterization at various stages from its initial synthesis to its final use. Moreover, the correlation of quantitative physicochemical data with the results of blood-material interaction testing and/or behavior in a device, which provides a rational basis for device development, can be considered valid only when material with similar properties, similar history and of a similar form is tested in each case.

TABLE 1. Tests Recommended for Physicochemical and Biological Characterization of Materials

	Bulk Properties	Surface Properties	Biological Response
LEVEL I	Polymer identification Infrared spectrum NMR spectrum Hardness Density Stress-strain relations	Light microscopic morphology Infrared spectrum Contact angles (limited)	Agar overlay tissue culture Agar overlay (extracts) Intradermial irritation Inhibition of cell growth Sterility assurance Endotoxin testing
LEVEL II	Solvent response Elemental composition and extractables Molecular weight distribution Extractability Thermal characterization Wide angle X-ray spectrum Optical birefringence Morphology (SEM) Electrical conductivity Creep Stress relaxation Oscillatory response	Morphology (High resolution, SEM) Contact angles (Zisman plot) Surface chemical composition - XPS Protein adsorption	Tissue culture (Liquid assay) Cell growth on solid surfaces Induction of hemolysis Intramuscular implantation (rabbits) Tissue culture (liquid assay) (extracts) Erythrocyte osmotic fragility Isolated rabbit heart test Systemic toxicity (mice) *In vitro* mutagenicity Sub acute intramuscular implantation Chronic intramuscular implantation

Accuracy, reproducibility, and interpretability are, in the opinion of the working group, the key elements in the value of any measurement. These depend not only on the method employed, but also on the equipment used and the investigator's skill and experience. To establish intercomparibility of measurements in various laboratories and reliable calibration standards for use in each laboratory, the working group is recommending the development of a series of reference materials which are also discussed in a following section of this report. We believe that these materials will not only provide calibration standards, but will have further utility in the future in providing material on which the usefulness of new characterization techniques can be demonstrated.

An important question which we cannot answer at this time, but which we believe should be addressed by the program, is the cost of materials characterization. It would appear appropriate, and it is our recommendation, that the program contract to one or more laboratories to perform the entire battery of tests outlined below on the reference materials, in order to fix the incremental costs of increasingly sensitive levels of characterization.

Finally, it should be noted that we have not provided any recommendations at this time on protocols for testing the effects of in situ fatigue and environmental stress factors on materials. The working group believes that this is an important area, but one which still presents great difficulty and must be studied further before standardized testing protocols can be recommended. The questions of appropriate ambient test conditions, of appropriate test fluids, of test duration are not now settled. The feasibility of accelerated testing is open to question since controllable variables in the acceleration, such as temperature and cycling rate, have different effects on mechanical and chemical properties of the materials. As a consequence, it is not possible to scale those variables in such a way as to affect both kinds of properties in a similar way.

For these reasons, this has been identified as a gap area at the present time. Studies are under way in several contracts supported by the program to understand the effect of environmental and mechanical stress factors and the working group endorses the continued support of such efforts. As information becomes available from these studies it may well be possible to offer guidelines on appropriate methods for assessing these factors.

CHAPTER III

BULK CHARACTERIZATION

Introduction

Bulk characterization of polymers is important for four reasons: It provides assurance that the material in hand is, indeed, that which the user expects it to be; it provides a basis for interpreting the results of surface analyses; it may give an indication of the effect the material is likely to have on blood; and it provides parameters that may correlate to the mechanical properties of the material.

As indicated in chapter II, the test procedures to be described are divided into three levels. The level I tests constitute the absolute minimum necessary for the material user to identify the polymer being investigated and to verify that the material is in accordance with the description provided by its supplier. These procedures are simple and any laboratory involved in the development of materials or in the construction of cardiovascular devices should have access to resources for such testing.

Level II tests are more appropriate for materials development. They are employed for materials which, on the basis of preliminary toxicological and blood-material interaction testing appear potentially useful. They provide detailed information on those elements of structure and functional behavior which are likely to relate to the performance of a material in a cardiovascular device. In all cases, the techniques described are capable of providing reproducible and interpretable information, although, in some cases, the sophistication of the techniques may require equipment and expertise available in only a relatively small number of laboratories.

Level III tests are those which are still in a developmental category. They are not presently useful for materials characterization, but represent directions for future development where further information on bulk properties appears necessary to explain or predict the performance of materials in devices.

In this chapter, bulk characterization procedures are described with particular attention to the rationale and limitations of each. For experimental details, literature references are provided.

LEVEL I TESTS

Polymer Identification

Because of the difficulty of retrospective analysis of polymer preparations, the most direct and economical way to understand the bulk properties of a given preparation is to have information on the precedures used to prepare it. For this reason, the group that actually polymerized the material is best informed about the nature of any specimens obtained from it. Whenever possible, information should be obtained from them before any analyses are undertaken. The information should include the following:

1. A statement of the monomers used in the preparation, and the methods employed in monomer and polymer purification.
2. The general method of polymerization used (i.e., radical, ionic, bulk or emulsion, kind of catalyst, concentration of components, reaction conditions such as temperature, pressure, timing) so that some assessment can be made by the characterization laboratory of the probable distribution of monomer assembly.
3. If the synthesis involved special efforts to control the polymerization, such as the use of transfer agents, the incorporation of branching points, etc., these should be identified. Similarly, if the structure is three-dimensional, the method used to achieve cross-linking should be specified.
4. Solvents used in the purification or isolation of the polymers often are the source of residues found in the bulk material. Both the kind of solvents used, and the quality should be specified, so that the evaluation laboratory can work with an understanding of the possible contaminants that may be present. Preferably, a solvent blank should be kept by the polymer preparation laboratory until level I and level II characterization tests have been completed.

Infrared Spectroscopy

To provide confirmation of the polymer sample data submitted with the specimens, an infrared spectrum of the bulk sample should be obtained (1). Infrared spectrometers of relatively low resolution should be adequate for this purpose and may be expected to be available in all laboratories involved in materials development and processing. Measurements can be carried out either on powder samples in pellet matrices, or in a suitable solvent for the polymer. Techniques for obtaining IR spectra will confirm the presence of the expected functional groups, and will serve as references for the surface characterization procedures described in chapter IV.

NMR Spectroscopy

Nuclear magnetic resonance (NMR) spectra can be readily obtained on soluble, non-cross-linked polymers (2), although the required instrumentation is relatively expensive and it may be necessary to contract with a commercial or university laboratory to perform this analysis. The data provide information on the chemical structure of the product, and some data as to its conformation. For copolymers it is the first information on possible sequences in the material. Furthermore, it will provide information on the presence of gross contaminants which are not readily separated from the polymer by fractional precipitation purification methods.

Hardness Parameter

The hardness of the material should be characterized in terms of a Durometer reading (3). In this test, the force required to produce a given indentation in a small ($\sim 1\ cm^3$) solid sample of the material is measured using one of several inexpensive commercially available devices. The thermal history of the sample will affect the test results, therefore, the thermal history should be provided with the test specimen to facilitate interpretation. Not only does this provide information on an important functional property but, as a measure of the compression modulus, it can be correlated with the presence of plasticizers in normally rigid or glassy polymers.

Density

Density measurements on solid polymers can be made quite easily by water displacement methods. They provide a simple means to compare specimens with respect to their bulk properties. Variations in crystallinity of solid plastics, void volume in cast polymers, or changes in loading of filled plastics are all reflected in small changes of density. Thus this simple measurement may serve as a screening test for comparison of different specimens representative of the same general class of test materials.

Bulk Mechanical Properties

The minimal requirement for characterization of mechanical properties is to obtain the tensile stress-strain curves of the materials of interest at low, medium and high rates of strain. This measurement is made on one of the standard tensile testing apparatuses, either floor or table model. The ASTM standard method for tensile testing is given in ASTM D-638 (4). The specimen, in the form of a "dog bone" or other strip shape is prepared by injection molding or cutting from a plaque of material. It is usually 4-5 cm long and has a cross-section sufficiently large that the extensional forces imposed can be controlled and measured. It is important not to have any notches, nicks or cracks on the test specimen. Both ends of the specimen are clamped firmly in the jaws of the tensile machine. The jaws are moved apart at the desired constant rate. Force, measured by a load cell, is automatically recorded vs. elongation. This may, of course, subsequently be converted to engineering stress (force/original cross-sectional area) or true stress (force/instantaneous area) vs. strain.

Figure 1 shows a generalized tensile stress-strain curve embodying the features usually seen in polymeric materials, and defining various mechanical quantities. The Young's or tensile modulus (E) (sometimes loosely called the stiffness), is the initial slope of such a plot. The area under the stress-strain curve is a measure of the total energy input required to fracture the material, and therefore, of the toughness of the material.

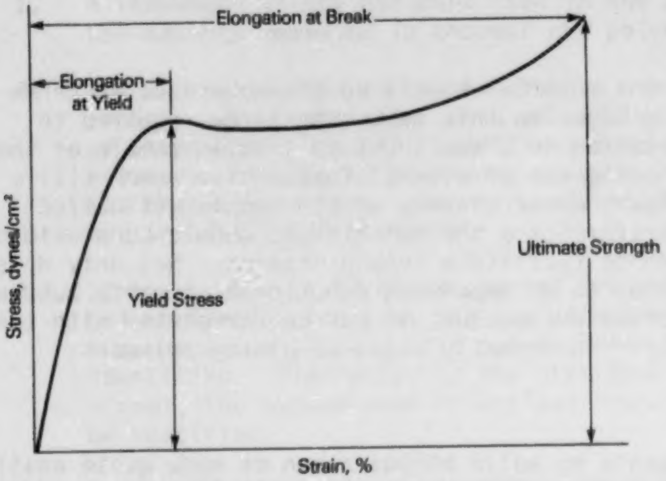

Figure 1. Typical stress-strain curve for plastics. From "Polymeric Materials" by Winding and Hiatt (5). Copyright 1961 by McGraw-Hill Book Company. Used with the permission of McGraw-Hill Book Company.

Strain rates in the range 1 to 1,000 percent/min cover the usual range. Strain rates of 1 percent/min, 100 percent/min and 1,000 percent/min would be suitable as low-, medium-, and high-strain rate choices. Figure 2 shows typical effects of variations in strain rate (ε_R) and temperature (T) on the observed stress-strain behavior.

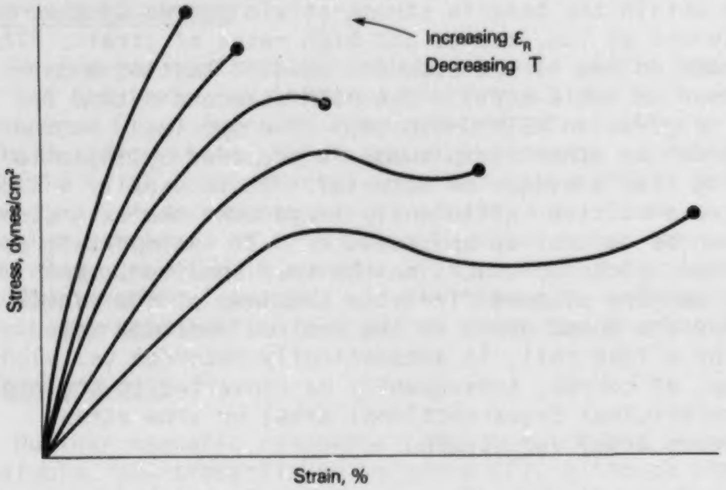

Figure 2. Affect of variation of strain rate (ε_R) or temperature (T) on stress-strain curve (6).

The shear modulus (G) of a material is related to the Young's modulus (E) by the relation:

$$G = \frac{E}{2(1+\mu)}$$

where μ is Poisson's ratio:

$$\mu = \tfrac{1}{2}\left[1 - \frac{1}{V}\frac{\partial V}{\partial \varepsilon}\right],$$

a measure of the dilation of the material during tensile strain. V is the test sample volume and ε is the strain. For incompressible materials, $\mu = 0.5$, and $G = \frac{E}{3}$. This is roughly true for lightly cross-linked elastomers but is, in general, not true for most plastics or for filled elastomers. The minimum value of μ ordinarily observed for homogeneous isotropic materials is about 0.2, but may be smaller for certain heterogeneous materials such as sponge rubber (7). Values of μ for polymers are generally nearer to 0.5 (incompressibility) than those for metals.

Thus, if Poisson's ratio for a material is known, or can confidently be taken as not significantly different than 0.5, then the shear modulus is obtained directly from a tensile experiment. The tensile device can be used with specially devised clamps to measure shear modulus directly. Measurement of shear modulus, however, is not recommended as an additional level I test since little new insight into material behavior is obtained.

Summary

The level I procedures will confirm the identification of the material structure and provide some indication of how the material is likely to respond chemically. The hardness and density measurements will, in addition, provide quality control indices. While their absolute values are not intrinsically meaningful, once an expected, or control value is determined, they provide a sensitive indication of small variations in samples nominally synthesized in the same way.

It is unlikely that the suggested tests will provide sufficient data to describe the material completely, since retrospective analyses of preparatory methods are always difficult and sometimes impossible. If the information on preparatory methods is not available from the material synthesizer, the extraction and chemical analysis procedures described under level II testing would be necessary.

The stress-strain data will provide a rough estimate of the strength and flexibility of the material, and they are especially useful as a method of intercomparison among materials. The use of varying strain rates will provide an initial indication of the time dependent response of the material, but will do little to elucidate creep properties and, indeed, more complete time-dependent response will require level II testing.

LEVEL II TESTS

Solvent Responses

An understanding of the solubility of polymeric materials to be used in cardiac assist devices is required for two reasons: (a) to assist in the selection of correct fabrication and cleaning techniques, and (b) to provide a description of the chemical bonding potential of the polymer. The first reason is a pragmatic one, and the second may become a useful adjunct to correlate the interaction between polymer structures and serum proteins to which they may subsequently be exposed. The determination of solvents which produce maximal swelling in cross-linked polymers can provide information on their segmental length and on their chemical bonding characteristics.

The description of polymer solubility suggested here on both applied and predictive grounds is based on the Hansen (8) modification of the Hildebrand (9) solubility parameter. The Hansen classification system uses a three-component solubility coefficient to describe the chemical bonding property of any potential solvent. The three components reflect the 1) hydrogen bonding, 2) permanent dipole-permanent dipole interaction, and 3) non-polar interaction characteristics of each solvent species. The hydrogen bonding component of the solubility parameter probably includes other "association" bonds; however, the historical use of this term favors continued usage for the purposes of this application.

The solubility parameter tabulations of Hansen can be related to the single valued parameters of Hildebrand through the following equation:

$$\delta_T^2 = \delta_H^2 + \delta_P^2 + \delta_D^2$$

where the subscripts H, P, and D, refer to the hydrogen, polar and dispersion bonding contributions to the coefficient. The solubility parameters for mixtures are additive on a volume fraction basis. Moreover, they are relatable to surface energies; Beerbower (10) has correlated the surface free energies of many organic liquids, fused salts, and liquid metals with the equation:

$$\gamma = 0.0715\ V^{\frac{1}{2}}\ [\delta_D^2 + 0.632(\delta_P^2 + \delta_H^2)]$$

Polymers are not described by solubility parameter point values, but by volume elements in a three-dimensional space defined in terms of the three coefficients. The boundaries of the volume element defining each polymer are determined by the location of those organic compounds which act as solvents for the polymer, i.e., polymer solvent coordinates fall within the space defined by the polymer, and nonsolvents are located outside of this volume element, (see figure 3).

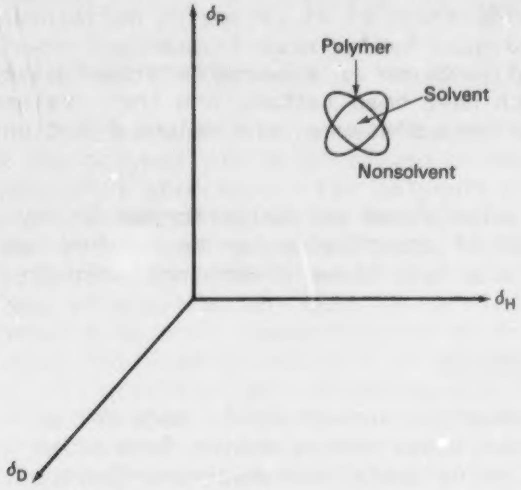

Figure 3. Solubility parameter space.

Because the differences in polarizability of many polymers are restricted, Hansen found that two-dimensional representations using only δ_H and δ_P are generally adequate. Using this two-dimensional notation, the polymer solubility is represented by the area encompassing the coordinates of its solvents. Nonsolvents have solubility coefficients whose coordinates are outside of this area. Solvent mixture coordinates prepared from two or more non-solvents for the polymer can be calculated on the basis of the volume fraction additivity of each component's coordinates. Similarly, interaction between the polymer and any organic species can be deduced from the distance between the polymer solubility boundary and the coordinates for that compound. The application of this concept to protein interaction has not been tested, but the concept is established for predicting plasticizer effects (11), polymer compatibility (12), and the effects of solvent removal on film formation (13).

Determination of solubility parameters is an approximate function but may be useful as a diagnostic tool. Determination of polymer solubility parameters does not require special sample preparation, except to ensure that the specimen is free of extraneous solvents/nonsolvents. The specimen need only be large enough to allow rough observations of dimensional changes. The measurements consist of equilibrium determinations of solubility at room temperature, and at elevated temperature in the case of semicrystalline polymers. Solubility is not quantitated; a determination of whether the specimen is soluble, swollen, or insoluble at 10 percent w/w is generally adequate to establish the profile. The data are reported in terms of the solubility coefficents provided by Hansen (8). A sufficient number of test solvents must be used to establish the boundary of the polymer coordinates. Where necessary, solvent mixtures are used to provide additional data points. When data on new materials are being compiled, similar studies should be performed on a reference material whose characteristics have been previously described.

The data should be presented in terms of a two-dimensional plot indicating those coordinates which have been tested, and the values assigned to each solvent. For solvent mixtures, the volume fractions should be reported.

The test procedure is quite simple and can be performed in any chemical laboratory. Computations of coordinates can be carried out with pocket calculators, even if the full three-dimensional coordinates are used.

Elemental Composition and Extractables

Contaminants in solvents, reagents, and catalysts used for polymer synthesis as well as adventitious contamination from polymerization vessels storage or shipping containers may contribute impurities to a final product. Since such impurities may be extractable and may eventually interact with the physiological fluids, it is desirable to determine their presence in the polymers prior to use.

Sample preparation should be based on minimal handling techniques in order to reduce contamination potential in the characterization laboratory. Samples should never be touched directly, should be stored and manipulated in a clean environment, and should be subjected to a minimum number of manipulation and transfers between receipt and testing. However, for speed of analysis a finely divided sample is generally desirable. Where simple elemental analyses are adequate, the sample should be ashed in platinum vessels and the residues examined by X-ray fluorescence or some other multielement procedure to identify the presence of potentially toxic metals. Quantitation may require more sensitive procedures such as atomic absorption. The precise methodology should be left to the discretion of a competent analyst. Many commercial and university laboratories have appropriate equipment and expertise to perform these measurements. The method of standard additions should be employed for quantitation to compensate for possible matrix effects. In the decision to use a particular procedure, consideration should be given to the possibility that the contaminants are not uniformly distributed throughout the bulk of the sample. Thus, localized analytical procedures which do not incorporate a significant sample mass should be avoided.

The determination of extractable molecular components remaining in the polymerization product is not a routine procedure. The difficulty of each characterization is a function of the kind of polymer under investigation, and of the prior purification processes used by the synthesis chemist. As a general rule, it is preferable that a precise characterization of all the components of the reaction mixture be available. This narrows the analytical problem to those species known to be present, or their known reaction products. For example, in a solution polymerization process for the preparation of condensation polymers, it is preferable to have well characterized solvent and reagent data rather than to attempt to identify residual impurities still present in the final polymer.

In the absence of such information, one must know whether or not the polymer can be dissolved or melted, or if it is a three-dimensional structure. For polymers soluble in volatile solvents, the preferred procedure is to dissolve the sample in either pesticide grade or spectral grade solvents, followed by precipitation from dilute solution with a nonsolvent of similar quality. Solvent blanks of equal volume must be carried through the procedure to provide a control. Concentration of the remaining solvent/nonsolvent mixture followed by analysis of the residues can then be carried out. If possible, gel chromatography/mass spectrometry procedures are recommended, since these lead to both qualitative and quantitative results. However, infrared spectra and NMR spectra of the residue may provide equally useful information.

For polymers insoluble in the available high purity solvents, but with melting points significantly below their decomposition temperature the polymer can be extruded in filament form into an excess of organic solvent selected to provide the same qualities described above. The work up can then follow as previously discussed.

For three-dimensional polymers the extraction must rely on the use of slow leaching procedures which permit adequate time for diffusion of contaminants from the bulk material. For that reason, the specimens should be finely divided. Extraction should preferably be carried out with both a highly polar hydrogen bonding solvent such as ethanol and with a less polar solvent such as methylene chloride. Both of these solvents can be obtained in high purity and can be removed from the extraction fluid by clean, dry nitrogen. As indicated above, the final identification of contaminants can be done by a combined spectral and chromatographic procedure. The appropriate method will have to be judged on the basis of the particular situation.

Molecular Weight Determinations

Molecular weights of two-dimensional polymers provide important information on the presence of oligomers (which may compromise mechanical properties), on the course of the polymerization of the specimen, and on the problems which might be encountered during processing. The simplest techniques of estimating molecular weight are those based on viscosity. However, the viscosity-based value provides no information on the distribution of molecular weights, which is an important determinant of mechanical fatigue. Therefore, we recommend that whenever possible the molecular weight be determined by gel permeation chromatography. It is necessary to calibrate the gel columns using control markers of known molecular weight. The procedure has been automated and is available through commercial laboratories, as well as in many laboratories specializing in polymer synthesis.

For monitoring the batch-to-batch variations of lots of nominally identical preparations, it is satisfactory to determine specific viscosities of dilute solutions of the polymer at three concentrations. These measurements can be made in a capillary viscometer, such as the Ubbelohde viscometer. Sample volumes of 5 to 10 ml are required. These data together with an intrinsic viscosity calibration performed on a qualifying sample will provide excellent control values, reflecting the average molecular weight of each batch. More details on the method and on viscosity nomenclature can be found in the literature (14).

For three dimensional polymers, the molecular weight is not an important variable. More directly applicable is a measurement of the gel swelling of each polymer preparation in a highly solvent fluid. The latter can be selected from a knowledge of the solubility parameter space for the polymer. The gel swelling of a three-dimensional polymer indicates the degree of cross-linking attained in the synthesis or subsequent processing.

Extractability

These procedures are directed toward determining whether or not necessary components of the polymer formulation are extracted, a situation which could lead to premature failure of the material. Many polymers require plasticization, stabilizers, and various adjuvants to achieve their mechanical properties and to stabilize them against degradation. For example, the phthalate esters are well known components of PVC resins used in tubing. The loss of significant quantities of such esters from PVC, or of the antioxidants that scavenge HCl, would lead to premature failure. This problem is quite independent of any toxic effects which might ensue from the leaching of the plasticizer.

No simple extraction procedure would simulate the effect of extended exposure to physiological fluids. Serum contains not only electrolytes, but also a variety of fats, surfactants, and nitrogen-based compounds. For the purposes of these experiments the extraction fluid should be chemically uncomplicated to facilitate subsequent analyses. If analysis of the extract is not possible, an alternative procedure is to characterize the residual polymer structure for changes in mechanical properties or phase relationships. The route to be taken must be left to the discretion of a competent investigator. We present here examples of each approach.

An aqueous alkaline emulsion of oleic acid/sodium oleate containing 150 mM NaCl provides a medium which is capable of emulsifying extractable fat-soluble components, and which is suitable for subsequent analyses. Acidification of the emulsion with dilute HCl breaks the emulsion, and leads to a two phase system. The aqueous components will be present in the electrolyte solution and the oil-soluble components will be present in the oleic acid layer. The former can be evaporated, and the usual inorganic or organic analysis scheme conducted on the concentrated solution. If the oleic acid layer is esterified, for example with MeOH, the resultant solution can be separated by liquid or gas chromatographic techniques to facilitate identification of components extracted from the polymer.

If the suspected extractables are sensitive to the alkaline conditions required to maintain the oleic acid/sodium oleate emulsion, the sample can be extracted instead with an aqueous solution of sodium dodecyl sulfate or other equivalent surfactant. The subsequent analysis is then best carried out on the polymer itself, following extensive water extraction to remove the surfactant. Water extraction should continue until the analytical results are not affected by further water extractions. At that point surfactant removal is assumed to be complete but can be assured by appropriate analysis of the polymer for traces of surfactant. If the polymer has undergone loss of a component which significantly affects its mechanical potential, measurement of the torsional modulus glass transition temperature (T_g) (see next section) or extension to break (see below) will generally reflect such losses. If the polymer has undergone loss of an important chemical stablizer, this should be reflected in oxidative stability tests.

Thermal Characterization of Polymer

As stated earlier, very few polymers are used under conditions of thermodynamic equilibrium with their environment. Thus there exists the potential for each device to undergo slow alterations in its structure during its lifetime. These alterations are expressed sensitively in the thermal response of the polymer. In the following section we will suggest two kinds of tests, each of which is affected by the prior history of the sample. To the polymer physicists, such information will provide a description of the polymer's potential to undergo alteration during its life cycle.

Differential thermal analysis (DTA) applied to polymers consists of the measurement of the change of temperature of the polymer (compared to a reference) as it is heated from a temperature characteristic of its glassy state to a point above its melting point. With a constant power input to the sample, the change of heat capacity resulting from some phase change in the polymer leads to a deviation in the time-temperature plot compared to a material not undergoing such a transition. Thus a change in slope is interpreted as a change in heat capacity, and this is associated with a new degree of molecular freedom in the sample. When the polymer is heated to the melting point the quantity of heat absorbed is the sum of the energy required both to raise its temperature and to melt crystalline forms present. By measuring this energy loss for both well-annealed and quenched samples it is possible to estimate the crystallization potential of each sample.

The DTA technique is perhaps best applied to thermally processed semicrystalline polymers to determine the crystallization history of the sample. It is also extremely useful to detect the presence of residual solvent, if the solvent has a heat of vaporization significantly greater than the heat capacity of the sample. Should residual solvent be present, the sample can be heated in a vacuum oven to remove it and then retested to ensure that the properties measured are those of the polymer itself. The DTA technique is not the method of choice for measurement of glass transition temperature.

The (T_g) of a homopolymer reflects the temperature at which segmental mobility of the polymer, as expressed by its relaxation time, corresponds to the time scale of the measurement. For a polymer undergoing cyclic stress at ever-increasing temperatures, there will be an observed temperature at which a discontiunity occurs in the curve relating modulus to temperature. This temperature will also be one at which a sharp increase in the viscous damping of any applied cyclic force occurs.

Since these effects reflect the action of only segmental lengths of the polymer (consisting perhaps of less than 20 repeating units) a copolymer having distinct domains may exhibit more than one transition temperature. Such measurements are, in fact, the best evidence that independently acting volume elements are present in the polymer. The effects of temperature, or contamination by components from the polymer's environment, will alter the transition temperature as well as the magnitude of the damping. Thus the measurement of modulus as a function of temperature is an extremely sensitive descriptor of polymer morphology.

The experimental techniques suggested for the thermal characterization of polymers are well known and widely applied. Heat capacity is measured by differential thermal analysis, in which the temperature of the specimen is compared with a control material when both are heated at a uniform rate in a high thermal capacity heating block. Differential scanning calorimetry (DSC) measures the energy difference required to maintain the polymer at the same temperature as a control material, when both are heated at a uniform rate. Both DSC and DTA provide essentially the same information, i.e., the locations of discontinuities in the polymer's heat capacity vs. temperature plot.

The data for DSC or DTA are presented as exotherm/endotherm plots vs. temperature (figures 4 and 5). Calibration points must be marked since the detectors are subject to drift. For precise work the heats of melting of reference compounds having melting points near the transition temperatures should be included. This calibration permits quantitation of the thermal effects.

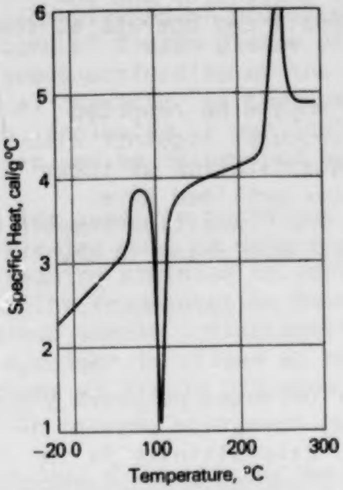

Figure 4. DSC analysis of amorphous poly(ethylene terephthalate). From "Textbook of Polymer Science" by Billmeyer (15). Copyright 1971 by John Wiley & Sons, Inc. Used with the permission of John Wiley & Sons, Inc.

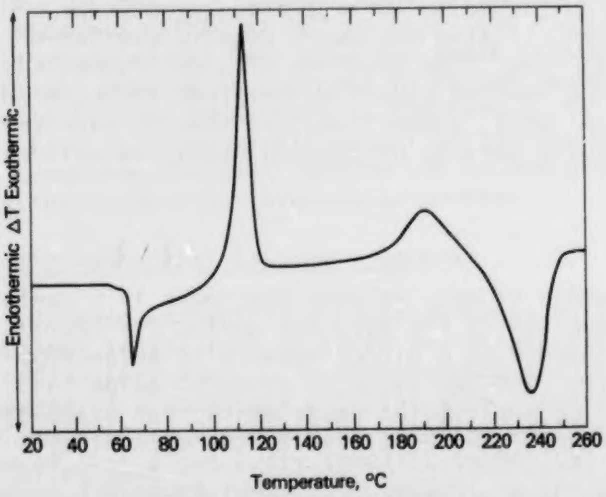

Figure 5. DTA analysis of amorphous poly(ethylene terephthalate). From "Textbook of Polymer Science" by Billmeyer (15). Copyright 1971 by John Wiley & Sons, Inc. Used with the permission of John Wiley & Sons, Inc.

The response of the polymer to stress as a function of temperature can be measured either with cyclical tension, as in a Rheovibron device, or with cyclic shear, as in a torsional braid analyzer. The torsional braid analyzer uses the polymer coated onto an inert glass filament so that the response up to and through the melting point can be followed. However, a section of the polymer itself can be subsituted for the braid if only low temperature data (i.e., less than the melting point) is sought. Both the Rheovibron and the torsional analyzers are temperature programmable and operate at low strain rates.

The mechanical response of the polymer should be reported either as shear or tension modulus vs. temperature, together with some measure of the damping observed. The quantitation of these measurements is not important for the purposes outlined here. Therefore, the only calibration required is the transition temperature, which can be established by some independent means in the device.

Wide-Angle X-ray Spectra

The X-ray spectra of either powdered or oriented polymers yield information on the kind and extent of crystal formation present in the sample at the conditions of measurement. Crystallinity is important not only for its possible effect on blood-material interactions, but also because of the dominant effects it exerts on mechanical responses of materials, and on the sorption and diffusion responses of the polymer to its environment. Because crystallinity is affected by a wide variety of variables reflecting the prior history of the specimen, it can be used to follow structural changes within a polymer as it is subjected to various processing steps. X-ray analysis requires sophisticated equipment usually available only in specialized laboratories. The spectrum of a well-annealed sample of the material being studied should be obtained for reference purposes. Once this is available, comparisons can then be made to the spectra obtained following various processing steps, as well as after implantation or use.

Optical Birefringence

The optical birefringence of many polymer specimens is a useful indicator of orientation applied to the specimen during its fabrication steps. The birefringence is a direct measure of anisotropy of the structural properties, and may serve as a useful guide in orienting the specimen during application experiments. For example, in membranes, the birefringence can be related to the mechanical moduli in the casting and cross-casting directions. In many solid parts, birefringence can be used to detect stresses frozen into specimens during fabrication.

Equipment to measure birefringence is simple and widely available. A petrographic microscope is required, and either a half-wave plate or a calibrated wedge. In the latter case, the wedge is calibrated in terms of the optical retardation of one plane of polarization compared to its perpendicular rays. The total retardation is a product of optical path length (i.e., specimen thickness) and birefringence.

Scanning Electron Microscopy (SEM)

SEM viewing of cross-sections of polymer specimens obtained by fracture of frozen pieces offers the investigator a means to establish the gross uniformity of the material and a measure of the morphological character of the specimen. Phase separations, void structures, inclusion of particulate contaminants, and distribution of fillers can be determined by this technique.

The specific technique used in the preparation of the sample for viewing must be determined for each specimen type. Some polymers may require staining in addition to the overcoating of a conductive film. The fragmentation method used can affect the morphology of the observed sample. Elastomers with very low T_g may undergo flow if the specimen is sliced at room temperature. For many specimens, fracture at liquid nitrogen temperature avoids most artifact formation.

Electrical Conductivity Measurement

Electrical conductivities of polymers can and have been measured by many techniques (16). They all involve some direct measurement of the resistivity (reciprocal conductivity) of the material when placed in a series circuit. Typical values for polymers span the range 10^{-14} to 10^{-9} $(\Omega \text{ cm})^{-1}$, although research toward conductive polymers has now produced values of 10^{3} $(\Omega \text{ cm})^{-1}$ and higher. The Shallcross milli-ohmmeter has been used for these measurements. The most difficult problem to overcome in making reproducible measurements, especially from laboratory to laboratory, is that of establishing good (low resistance) electrical contact between the material and the measuring circuit. ASTM-D-150 is the standard test for insulating properties (dielectric constant).

Dynamic Mechanical Testing: General

Dynamic mechanical testing consists of two classes of tests: transient tests, such as creep and stress relaxation, and periodic or oscillatory tests. To characterize the full range of dynamic response of a candidate material with respect to frequency (or time) and temperature dependencies requires utilization of more than one type of test since every specific mechanical testing device has a limited frequence range with respect to the range of interest. Ferry's text (7) is the best single reference for information on this type of testing, and is especially thorough in describing the interrelations among the material functions obtained by various dynamic tests done within the linear response range of the material. The level I tests should be completed before initiating level II testing since some preliminary information is necessary. These level II tests in many cases require somewhat more specialized equipment than that required in level I.

Creep

The creep experiment consists of applying a stress (tensile or shear) to a sample within a very brief time (t=0) and maintaining it constant thereafter. The time-dependent strain of the sample is measured. Typical plots of stress (σ) and strain (ε) are shown in figure 6. The property measured is the creep compliance, D(t), defined by:

$$D(t) = \frac{\varepsilon(t)}{\sigma_0}$$

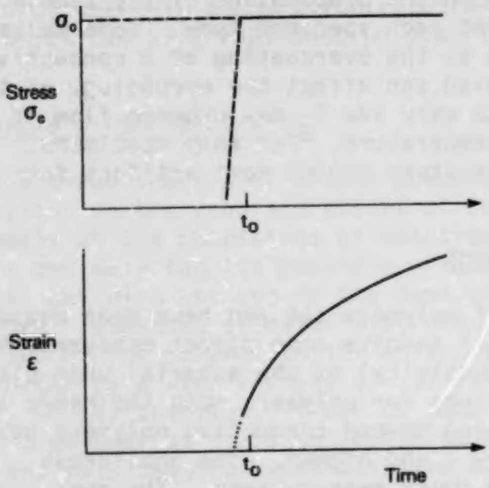

Figure 6. Demonstration of creep. At a constant stress (σ_0) the strain (ε) of a viscoelastic material will increase with time.

For cross-linked materials the strain, ε, will reach some steady-state value (ε_s). For uncross-linked materials, the strain will continue to increase until failure, often in such a way that a steady-state flow develops and the strain as a function of time may be written:

$$\varepsilon(t) = \sigma_0(D_e^0 + t/\eta)$$

where η is the viscosity of the material (extensional if measured in tension) and D_e^0 is a measure of the elastic recovery of material known as the steady state recoverable compliance. This is also the ratio of the amount of recoverable strain to applied stress determined by rapidly unloading the sample. Figure 7 illustrates these concepts.

Because of the simplicity of the creep experiment, a wide variety of arrangements can and have been used. The simple way of applying the force is by means of a dead weight; displacement is conveniently measured by a differential transformer, traveling microscope or cathetomer, or deflection of a light beam.

For comparatively soft materials, sample gripping may be difficult. Torsional shear creep measurement in an apparatus such as that described by Plazek (17) is recommended. For materials with tensile moduli greater than 10^7 Pascals, the tensile creep mode is most convenient, but because of the large σ_o which must be applied a rather massive testing instrument will be required. The Instron Tester is a versatile instrument capable of automating the creep experiment for stiff candidate materials.

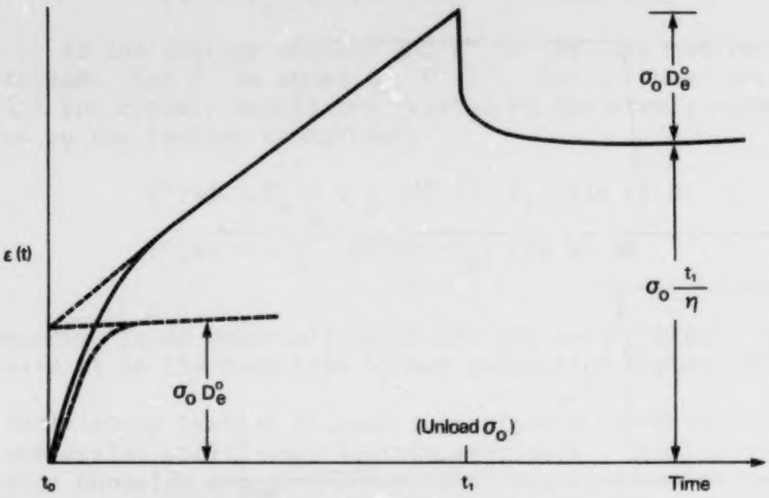

Figure 7. Permanent and recoverable creep.

In all tensile testing modes corrections will have to be made for the changing cross-sectional area. Constant σ_o can be maintained by reducing the applied force continuously. Temperature dependence of the creep compliance should be studied by performing the measurements in a temperature-controlled environment. Such environmental chambers are commercially available.

Stress Relaxation

The stress relaxation experiment consists of rapidly straining (tensile or shear) the sample to some constant strain σ_o and subsequently monitoring stress as a function of time. Typical plots of strain and stress are shown in figure 8.

The material function measured is the stress relaxation modulus, E(t). Experimental methods and procedures for stress relaxation have been reviewed in detail by Bergen (18). Most measurements are made in extension. There are, in general, three ways of keeping strain constant: by continuous adjustment of the force as the stress in the sample relaxes using an automatic servomechanism; by applying more than enough force with a stop to limit the deformation; and by straining a stiff spring element in series with the sample.

When stress relaxation experiments are performed on uncross-linked polymers, the stress eventually decays to zero in a way that can be fitted by a series of exponentials to give an approximate analytical expression for the time dependent stress relaxation modulus, i.e.,

$$E(t) = \frac{\sigma(t)}{\varepsilon_o} = \sum_{i=1}^{N} E_i \, e^{-t/\tau i}$$

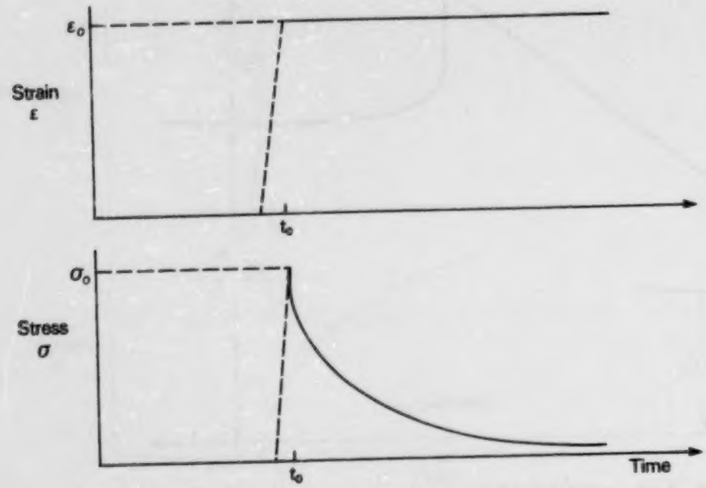

Figure 8. Demonstration of stress relaxation. At a constant strain (σ_0), the stress (ε) of a viscoelastic material will decrease with time.

where the τ_i are relaxation times for the material. Usually only two or three of the τ_i can be extracted from the stress relaxation data directly with any claim to accuracy. The small forces must be measured with a sensitive detector toward the end of the experiment. With cross-linked polymers the stress approaches a limiting value and it is necessary to determine small changes toward the end of the experiment. Several stress measuring devices with different sensitivities should be available for use in this situation. For stiffer samples, commercial testing devices can automate the stress relaxation experiment and provide a temperature-controlled environment.

Oscillatory Testing

The protocol here is to subject a sample of the test material to an oscillatory strain and observe the oscillatory stress response. If the applied strain has the form

$$\varepsilon(t) = \varepsilon_o \, e^{iwt} = \varepsilon_o \, (\cos wt + i \sin wt)$$

it is found that for a viscoelastic material in the linear response range, the stress response may be written:

$$\sigma(t) = \sigma_0 \, e^{i(wt + \delta)}$$

where δ is the phase lag of the stress expressed as an angle. The stress may be rewritten, defining two material functions, as:

$$\sigma(t) = \varepsilon_0 \, [E' (\sin wt) + E'' (\cos wt)]$$

where E' is the storage modulus and E" is the loss modulus. The loss tangent, tan δ is equal to E"/E'. For a linear viscoelastic material the dynamic moduli are related to the stress relaxation modulus by the Fourier transforms:

$$E'(w) = E_e + w \int_0^\infty [E(t) - E_e] \sin wt \, dt$$

$$E''(w) = w \int_0^\infty [E(t) - E_e] \cos wt \, dt \; .$$

For uncross-linked materials which are not very viscous, E_e is zero; otherwise it is the long-time stress relaxation plateau modulus.

Oscillatory testing is most conveniently performed with one of the many commercial oscillatory testers available. Tensile oscillation, torsional pendulum and forced torsional oscillation are the most common measurement modes (the latter two giving dynamic shear moduli G' and G"). With a temperature-controlled commercial apparatus operating in the tensile mode, moduli and tan δ are obtained directly. Disadvantages of such an apparatus are: 1) the possibility of background stress relaxation due to a small tensile strain which must be applied to prevent buckling during testing and 2) the limited number of fixed frequencies available. Advantages include the ease of operation and the low cost which makes it feasible in many laboratories.

The torsional testing mode is superior from the testing point of view. A forced torsional oscillation device with environmental control for the mechanical spectrometer is available. Such instrumentation has a continously variable frequency range but the high cost (∿$100,000) makes it less accessible to many laboratories.

Both types of dynamic mechanical testing instruments require strips of the material. If the material is very soft, serrated platens can be used to hold and subject a thin disk to torsional oscillatory strain in the mechanical spectrometer.

From this mode of testing one obtains the dynamic moduli as a function of temperature and frequency. There is a correspondence between the temperature and frequency dependence of material functions for many polymers which is expressed analytically by the Williams-

Landel-Ferry (WLF) equation (19):

$$\log a_T = \frac{-C_1(T-T_g)}{C_2 + T-T_g}$$

where a_T is a so-called WLF shift factor. This factor is the amount by which mechanical property data obtained at temperature T [E(t), D(t), E'(w), E"(w), etc.] must be shifted horizontally along the time (or frequency) axis with respect to data obtained at some reference temperature (here taken to be the glass transition temperature, T_g) in order to form a so-called "master curve." The constants C_1 and C_2 are empirically determined for every material. A construction of such a master curve is illustrated in figure 9. Similar master curves can be produced for any linear viscoelastic material function.

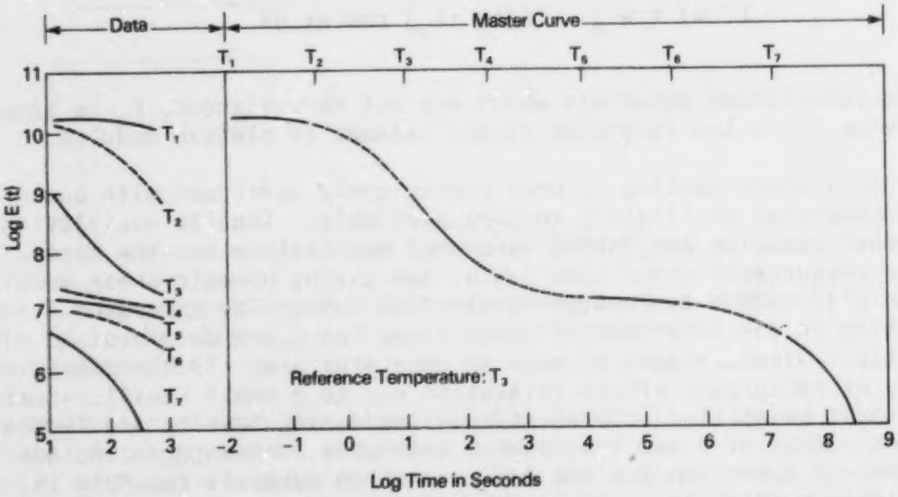

Figure 9. Development of a stress relaxation master curve from experimentally measured modulus-time curves at various temperature. From "Introduction to Polymer Viscoelasticity" by Aklonis, Macknight and Shen (20). Copyright 1972 by John Wiley & Sons, Inc. Used with the permission of John Wiley & Sons, Inc.

Such data on temperature dependence have been tremendously valuable in studying glass transition and other structural transitions in the amorphous and crystalline phases of semicrystalline polymers. The frequency dependence gives important information on how the material responds to rapidly applied deformations (high frequency) relative to more slowly applied deformations.

Summary

In level II tests of bulk properties, the time dependent response of the material is probed more deeply in the linearly viscoelastic response regime. It is recommended that at least one transient and one oscillatory type measurement be made, both as functions of temperature. The former is more effective in probing the long time response while the latter is vital to understanding the high frequency response. With proper choice of experiments, the combination can be used to give information over 10 or more decades in log time.

This level II information has been used by Smith (6) and by Halpin (21) in studying the ultimate properties of polymer materials. In particular, Smith has shown how a "failure envelope" can be constructed from stress relaxation and creep data.

LEVEL III TESTS

Solute Mobility

The mobility of a solute in polymers proposed for various applications in cardiac assist devices is an important factor both in the synthesis of the material and in its behavior in a device. Mobility of solvents and of other processing aids in the polymer must be great enough to permit total removal of these species when a component is solvent cast so that the components are contaminant-free. Low mobility of solvents through a polymer can lead to retention of the solute as an impurity and its slow release over a period of years may lead to toxic responses quite unrelated to the basic polymer chemical properties. Thus, high mobility of fabricating solvents is desirable.

On the other hand, high mobility of physiological fluid components in the polymers of the device in contact with blood or other fluids may lead to loss of physical properties through plasticization, induced crystallization, or alteration of phase relationships. Thus an understanding of the relative mobilities of compounds, classified according to their potential interaction with the polymer, will be a necessary factor in predicting the useful life of cardiac assist devices.

The mobility of physiological or chemical solutes in polymers has not yet been studied widely in connection with blood-material interactions; therefore, the working group considers this to be a developmental area. However, there are a number of guidelines for such studies.

No single set of test solutes will provide the necessary mobility data for every polymer potentially useful in cardiac assist devices. Selection of suitable test solutes for each polymer can made once the two-dimensional solubility parameter coordinate system has been established for the polymer (see above). Using this coordinate system, it is possible to select solutes from the solvent (coordinates within the solubility area) and nonsolvent (solutes with coordinates outside the solubility area but between those of the polymer and water) groups. At least one solute should be selected from each category.

Mobility data using the solute representing the "solvent" category of compounds will provide data on the removal rates of residual solvents following processing. If solvent mixtures containing a nonsolvent are used in processing, the test should be performed with the poorer solvent. If the polymer is hydrophilic, the coordinates of the wet polymer must be determined.

In order to study the mobility of physiological solutes, the solute from the nonsolvent category should be representative of the types of solutes found in blood or plasma, i.e., lipids, steroids, amines, etc. Butyl alcohol is one solute which could serve to represent the amphipathic nature of many biologic compounds. The use of butyl alcohol also offers the potential for correlation with the usual pharmacologic characterization of drug distribution in body fluids.

The techniques for measuring mobility suggested here are quite simple, and rely on the use of standard water transmission rate cups (22) in a vacuum chamber. The cups clamp film samples across a reservoir opening. When operated upright, only the vapor from the cup contacts the polymer specimen. When operated in an inverted position, the entire fluid contacts the polymer sample. The two results are generally not equivalent: the vapor transmission rate is for the polymer in equilibrium with the partial pressure of the permeant component; the liquid permeation rate is for a polymer swollen with the liquid phase. Measurements are performed by storing the cups at 37° C in a vacuum chamber operated at a pressure sufficiently low (approximately 1 mm Hg) to provide a constant pressure difference corresponding to the partial pressure of the permeant at 37° C. For single component permeants, the analysis of mass transferred as a function of exposure time is sufficient to provide a permeation rate. For binary, or multicomponent permeant mixtures, the residual composition on the source side must be analyzed as a function of time. The time span of the experiment can be adjusted by use of thin or thick polymer films and by the selection of permeants with high partial pressures at 37° C.

Even for the solute outside the solubility zone of the material, the material should be exposed to both the vapor and the liquid phases. The permeation rate of an organic solute is often quite different in the presence or absence of water even if the polymer was hydrophilic in character.

The data should be reported in terms of the permeation rate as a function of time (or mass of solute transported vs. time). For some solute/polymer combinations it will be possible to establish the lag time period required for the permeant to achieve a steady state distribution in the polymer. These data will provide information on the diffusivity of the solute in the polymer. When specially selected solutes are used, some explanation for their selection should be given. For example, if polysiloxanes were to be tested for potential application as a bladder material, it would be appropriate to test the permeation rate of a fatty acid ester, such as methyl oleate, in order to find some predictive data for the permeation of apolar serum components.

References

1. R. Zbinden: Infrared Spectroscopy of High Polymers, Academic Press, N.Y., 1964.
2. F.A. Bovey: High Resolution NMR of Macromolecules, Academic Press, N.Y., 1972.
3. ASTM D-785, 1970 Book of ASTM Standards, parts 26 and 27, Plastics, ASTM, Philadelphia..
4. ASTM D-638, Op. Cit.
5. C.C. Winding, G.D. Hiatt: Polymeric Materials McGraw-Hill New-York, 1961.
6. T.L. Smith: J. Polymer Sci. A-1, 3597, 1963.
7. J.D. Ferry: Viscoelastic Properties of Polymers, Wiley, N.Y., 1971.
8. C.M. Hansen: J. Paint Technol. 39, 104, 510 and 555, 1967.
9. J. Hildebrand and R. Scott: Solubility of Nonelectrolytes, Rinhold, N.Y., 1949.
10. A. Beerbower: J. Coll. Inter. Sci., 35, 126, 1971.
11. C.M. Hansen and A. Beerbower: Kirk-Othmer Encyl. Chem. Tech., Supplementary Vol., 889, 1971
12. J. Brandrup and E.H. Immergut: eds. Polymer Handbook 2nd Ed. Wiley, N.Y., IV-337 1975
13. H. Burrell: ACS Div. Org. Coat. and Plastics Preprints, 31(2), 367, 1971.
14. J.B. Kinsinger: in Encyclopedia of Polymer Science and Technology, H.F. Mark and N.G. Gaylord, eds., Interscience, N.Y. Vol. 14, 717, 1971.
15. F.W. Billmeyer, Jr.: Textbook of Polymer Science, 2nd Ed. Wiley, N.Y., 122, 1971.
16. Symposium on Electrically Conducting Polymers. Bull. Am. Phys. Soc. 25, 1980.
17. D.J. Placek: J. Polymer Sci. A-2, 6, 621, 1968.
18. R.L. Bergen: in Testing of Polymers, J.V. Schmitz, ed. Wiley, N.Y., Vol. 2, 1, 1966.
19. M.L. Williams: R.F. Landel, and J.D. Ferry. J.Am. Chem. Soc. 77, 3701, 1955.
20. J.J. Aklonis, W.J. Macknight and M.C. Shen: Introduction to Polymer Viscoelasticity, Wiley, N.Y. 1972.
21. J.C. Halpin: J. Applied Phys. 35, 3133, 1964.
22. V. Stannett, H. Yasuda: in Testing of Polymers, J.V. Schmitz, ed. Wiley N.Y., Vol 1,393, 1966.

CHAPTER IV

SURFACE CHARACTERIZATION

Introduction

The surface characteristics of biomaterials are believed to be related to their blood-material interactions. It is commonly accepted that the protein adsorption characteristics of these materials are critical in determining their eventual blood compatability, including the adhesion of platelets, interaction with white cells, possible interaction with red cells, etc. It is becoming evident that blood protein-material interactions may involve components of the complement system, which may further interact with white cells, etc. Therefore, it is important to know something about the surface characteristics of the materials which are being used for cardiovascular devices and for the study of blood-material interactions. Although there has been considerable research activity over the past 20 years on blood-material interactions and some activity attempting to correlate the surface properties of materials with those interactions, the number of correlations which presently exist are very few indeed. Part of the problem is due to the fact that the blood-materials work in the past has largely involved the use of materials whose surfaces have not been adequately surface characterized, or, in many cases, not characterized at all. It appears obvious that if one is going to attempt to correlate blood-material interactions with the surface properties of a material, then one must at least be able to measure and characterize those very surface properties.

The three levels of testing described in chapter II are reviewed here with emphasis on their applicability to surface analysis and characterization. Level I consists of relatively simple test and characterization methodologies which can, in principle, be performed in virtually any laboratory involved with the development of biomaterials. They involve a minimum of apparatus and expense and can be performed with no specialized training. In many cases the materials information required in level I testing will be available from the supplier of the material.

Level II surface characterization assumes that, on the basis of level I testing, a material appears to be of interest for further study or development. Level II bulk characterization tests should already have been completed to provide information on the bulk chemistry and properties of the material. The level II surface characterization will provide relatively detailed information on the surface and interfacial properties of the material which are expected to be related to subsequent blood-material interactions. They involve relatively sophisticated equipment and, in some cases, equipment that would not be expected to be present in many research laboratories. However, all the tests suggested at this level can be performed in specialized, commercial analytical laboratories and are generally accepted analytical techniques with minimal interpretation problems. Investigators who do not have access to such specialized equipment in laboratories in their plant or institution may make arrangements to have the samples appropriately analyzed by other laboratories.

Level III tests are generally research techniques and methodologies and, in several cases, still unproven or of a somewhat qualitative or only semiquantitative nature. These tests and methods will be important in probing and understanding many of the gap areas or need areas discussed in this report. One example is the role of microheterogeneity in the surface characteristics of materials, particularly the block copolymer systems which are widely used as biomaterials, for example, the polyurethanes. The characterization of morphology down to 25 Å level is a very difficult surface analytical problem at present. It is expected that the level III characterization will only be attempted by those researchers active in the field of surface analyses of biomedical materials. It is also expected, however, that many of the tests in level III will be refined and developed in the near future for inclusion in the level II category.

This chapter includes a number of detailed descriptions of various surface properties and in some cases the descriptions of methodologies for obtaining the requisite information on surface physicochemical properties.

It is important to note that different materials can have dramatically different surface properties. In some cases tests suitable for probing a certain range of properties may be limited to only a certain class of materials. Nevertheless, the types of tests suggested for level I and level II are such that they can be expected to apply across the entire range and breadth of materials normally considered useful and of interest for cardiovascular device development and application.

LEVEL I TESTS

Introduction

Figure 10 presents a suggested flow diagram for the level I surface analysis of a typical medical material.

The surface of a sample should not be characterized unless the bulk characteristics of the sample are already known. Therefore, the level I bulk characterization discusses in Chapter III should be available prior to the level I surface characterization.

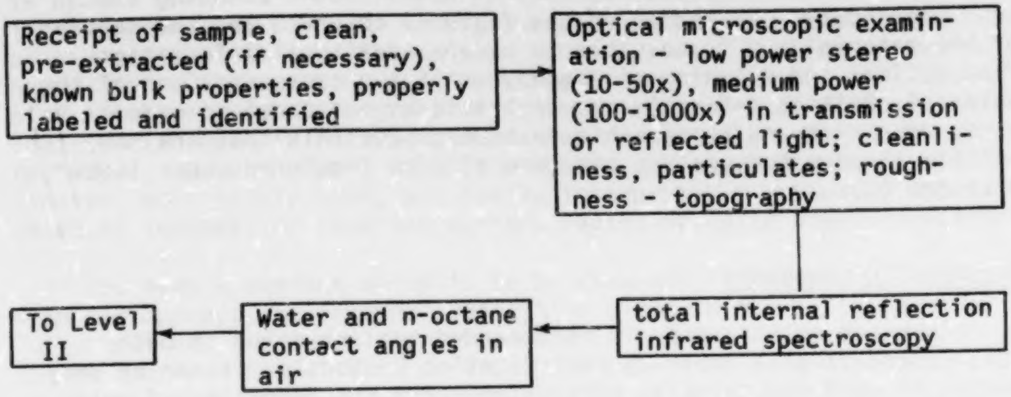
Receipt of sample, clean, pre-extracted (if necessary), known bulk properties, properly labeled and identified
Optical microscopic examination - low power stereo (10-50x), medium power (100-1000x) in transmission or reflected light; cleanliness, particulates; roughness - topography
total internal reflection infrared spectroscopy
Water and n-octane contact angles in air
To Level II

Figure 10. Recommended flow plan for level I surface characterization.

Microscopic Examination

All material, prior to any type of surface characterization, should be examined in an optical microscope for surface homogeneity or heterogeneity, the presence of particulate matter, surface defects, scratches, etc. This should be done with a low-power stereo microscope and then at relatively high magnification with a conventional optical microscope. For example, a surface roughness of the order of 0.1 μm or greater (1) will make it difficult to interpret any contact angle measurements made on the material. It is reasonable to expect that materials used for blood-material interaction studies would be largely particulate-free and relatively smooth and homogeneous. This means that the materials should be prepared and studied in a relatively dust-free environment, preferably in a laminar flow, filtered air laboratory bench or in a clean room.

A detailed discussion of particulate contamination and characterization is presented in appendix A. A further discussion of particulate contamination and sample handling is presented in Guidelines on Blood-Material Interactions.

The microscopic examination should pay careful attention, in addition to particulate matter, to the presence of surface roughness and deposits, including extrusion marks, machining marks, etc. The orientation of such features should be clearly documented. Orientation effects in many polymers can be clearly seen by the use of cross-polarized light microscopy (2).

Certain types of material surface features will be difficult to see because of the material's optical characteristics, unless one uses interference or phase contrast illumination or scanning electron microscopy. Depending on the features observed and the nature of the material, it is possible to obtain additional information from optical and scanning microscopy utilizing cross-sections of the material. Brittle materials can be fractured and examined edge-on to study roughness and topographic features; materials that are nonbrittle at room temperature can generally be fractured under liquid nitrogen (3).

Infrared Spectroscopy

Infrared spectroscopy is recommended for inclusion in both level I and level II bulk characterization protocols. It can be very useful in surface analysis, particularly for the detection of mold-release agents, surface-active agents, antistatic agents, and related additives, surface oxidation, and processing effects. By the utilization of a relatively simple total internal reflection accessory for virtually any infrared spectrometer, one can obtain an infrared spectrum of the surface region of nearly all solid polymers (4).

Internal reflection infrared spectroscopy (figure 11) allows the recording of the diagnostic infrared "fingerprint" for the surfaces of solids, liquids, and powders of thin films. The technique is sensitive enough to detect and identify some films as thin as 10 Å (where absorption bands are sufficiently strong), and to follow changes in the composition, configuration and bonding within such films. The method is nondestructive, and can be applied with few reservations to delicate materials.

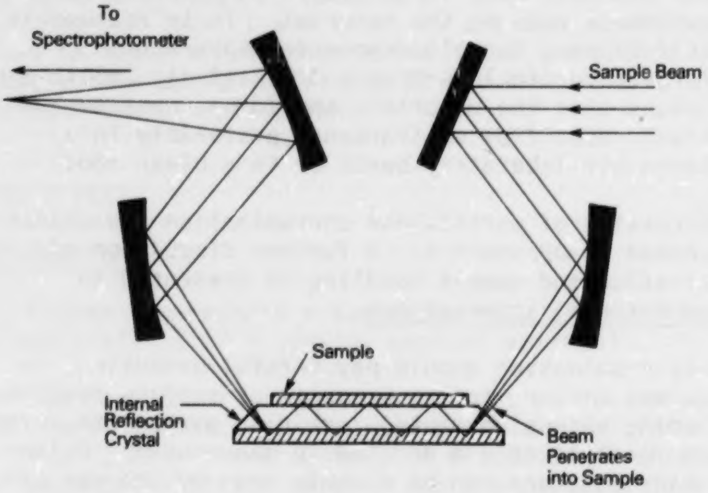

Figure 11. Typical geometry for total internal reflection spectroscopy.

It is important to note that even though the technique can be very surface sensitive, the penetration depth of the infrared beam is of the order of a wavelength (5), which is in the micron range for infrared spectroscopy. Thus, the technique really measures a considerable depth into the sample. In many cases surface contaminants that are present only in very small concentrations, portions of a monolayer, subtle degrees of surface oxidation, or surface species with weak IR absorbing bands may not be detectable by this technique. Nevertheless, this is probably the simplest, least expensive, most widely used, and easily interpretable method to obtain chemical information from the surface region of solid organic materials.

The sample surface which is to be examined is placed in contact with the appropriate internal reflection plate, pressure is carefully applied, and the plate is mounted in the apparatus and placed in the infrared beam. With care paid to reference beam attenuation an excellent infrared spectrum can usually be obtained which can then be analyzed by standard methods (3,4).

Contact Angles

A measure of the surface free energy and/or surface wettability of solid materials is most readily and simply obtained by measuring the contact angle of a diagnostic liquid on the solid surface. This is an extremely sensitive technique for identifying changes in surface composition (6). The contact angle is usually measured in the advancing mode (7). This method is suitable for most of the relatively common rigid polymers which cannot reorient in equilibration with their environment. A discussion of the critical surface tension concept is given in appendix B.

Before a contact angle measurement can be very meaningful it is important to guarantee that the probe liquid is not extracting soluble material from the solid surface or, conversely, that the solid is not swelling. Some test of extractable matter should be carried out, including a chemical assay of the extracted material. The materials to be examined by contact angles should be subjected to extensive preextraction to guarantee that the extraction characteristics do not interfere with subsequent materials characterizations or subsequent blood-material interaction studies. Small amounts of residual material which are extractable into the water droplet are generally surface active and would change the surface tension of the water, thus changing the contact angle (8). A "Teflon Test" can be used to detect such changes (9) (see appendix C).

For those polymers which have a significant degree of surface mobility and can readjust their surface region in response to their local environment, the contact angle measured in air or vapor is not generally satisfactory. More direct information related to polymer-water interfaces is obtained via the captive air or captive bubble technique (10). The polymer is totally immersed in water or in a suitable saline solution. An air droplet is then introduced via a curved microcapillary tube and allowed to rise to the polymer-water interface. The solid-water-air contact angle is then measured. In this case the polymer is fully equilibrated with water prior to the application of the air bubble, and the polymer-water-air contact angle is a measure of the fully hydrated polymer-water interface. This is actually somewhat related to what would be called a receding water contact angle in the common technique where the water droplet would be placed on the polymer in air. One can, in addition, use several water-immiscible liquids as probes to obtain further information about the polymer-water interface. A common probe that has been used for this purpose is normal octane. This technique may be particularly informative for materials with block or domain structures, one or both of relatively high mobility, where one domain can dominate at hydrophobic or air interfaces and the other may dominate at hydrophilic or water interfaces.

In level I characterization, both advancing and receding water and octane contact angles should be measured (11). These measurements can be made with a relatively simple apparatus. They are commonly made with a horizontal microscope of about 20-100x magnification and a small drop of water dispensed from a microsyringe. The drop volume is commonly about 1 microliter. The contact angle at the point of tangency can be directly measured with a goniometer eye piece or the drop dimensions can be measured and related to the contact angle by trigonometric functions. The drop can also be photographed and the angle measured with a protractor on the photograph. These and other methods are all described in basic textbooks and reviews on surface chemistry (such as 1,11).

The advancing angle is measured by slightly increasing the drop volume with time and measuring the angle; the receding angle is measured by slightly decreasing the drop volume with time. These measurements are a bit more difficult to perform. Generally, the initial drop placed on the solid represents the advancing angle. The angles are strong functions of surface roughness and any heterogeneity in the surface characteristics of the material.

Summary

The level I surface characterization protocol thus provides information on surface roughness, topography, morphology, and particulate contamination by means of the optical microscopy study. It provides information on the surface organic chemical functional group character, including the presence of surface contaminants and the presence of surface oxidation, by the total internal reflection infrared spectroscopy technique. It also provides information on the surface wettability and the surface energy as measured via the contact angle techniques. This information can be obtained in virtually all laboratories. All that is required is a simple optical microscope, a camera with a closeup lens for photographing a contact angle drop, or a horizontal microscope equipped with a gonionmeter eye piece. A simple infrared spectrometer equipped with a total internal reflection attachment is adequate for the infrared study.

LEVEL II TESTS

Introduction

The function of the level II surface characterization protocol is to obtain further information on the surface characteristics of materials which appear to be stong candidates for further development and for further testing for blood-materials contact studies or for cardiovascular device development. It is assumed that level I characterization has already been completed.

The increased sensitivity and improved information provided by many of these level II tests (and the level III tests described later) relate to the sampling depth and area of surface resolution. The "true" surface of a material--the region whose properties differ from those of the bulk of the material--extends into the material only a few molecular layers. Therefore, techniques which sample much more deeply may obscure surface data. Similarly, highly localized structure, associated with domains or with other surface heterogeneities, can be obscured or overlooked by a sampling probe that has poor surface resolution. Figure 12 summarizes the depth and spatial resolution achievable with a number of the techniques described in this and the following sections.

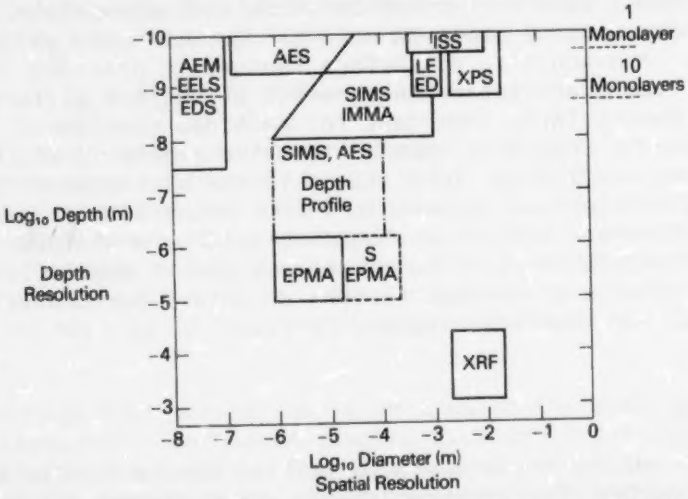

Figure 12. Depth and spatial resolution achievable with current analytical techniques, including x-ray fluorescence (XRF), electron spectroscopy for chemical applications (ESCA) [also known as x-ray photoelectron spectroscopy (XPS)], ion scattering spectroscopy (ISS), Auger electron spectroscopy (AES), ion microprobe mass analysis (IMMA), low energy electron diffraction (LEED), secondary ion mass spectroscopy (SIMS), electron probe microanalysis (EPMA) and scanning electron probe microanalysis (SEPMA), and analytical electron microscopy (AEM) including electron energy loss spectroscopy (EELS) and energy dispersive x-ray spectrometry (EDS, commonly referred to as EDAX).

Microscopic Examination

Optical microscopy of higher resolution than that used in level I tests can be carried out utilizing interference and phase contrast methods, polarized light methods, etc. Scanning electron microscopy should be seriously considered. The elemental nature of inclusions of hetrogeneities in the surface, particulate matter, etc., can be easily examined in the scanning electron microscope both morphologically and elementally by the use of the X-ray fluoresence analysis (XRF) mode commonly found in virtually all modern SEM's. However, in this approach, the material is sampled to a depth of a few tenths of a millimeter, so care must be taken in interpreting the results. Detailed descriptions of SEM and energy dispersive analysis of X-rays (EDAX) are available in the literature (12) and a variety of commercial laboratories provide such services at nominal fees.

An electron microprobe may also be employed in conjunction with SEM's (EPMA). It has the advantage of better energy discrimination and therefore better quanitative analysis of elemental composition. The surface texture of a material can be well characterized by SEM particularly by using stereo pairs and doing a surface topography analysis (13). Other methods of measuring surface texture include available profilometry techniques. These are quite difficult to do on soft specimens, such as typical polymers and appropriate controls and measurements must be employed when using techniques which rely upon contact. A discussion of surface texture is provided in appendix D. Some quantitative measurement of surface texture or roughness is particularly important for machined specimens. Because roughness elements create regions of relative stasis in fluids adjacent to the surface and such stasis in the vicinity of the blood-materials interface appears to increase the likelihood of clotting, texture and roughness should be held to a minimum, preferably at the 5 micron level or below, unless one is specifically studying the effects of surface texture or unless surface texture is being utilized for some advantageous purpose.

Contact Angles

Level II contact angle characterization should include measurements with a series of diagnostic liquids as described in the critical surface tension protocol outlined in appendix B. The liquids to be used must be carefully screened to ensure that they do not attack and are not imbibed by the sample material. The protocol should include the measurement of advancing and receding contact angles in order to obtain some measure of surface homogeneity and surface roughness. A good example of the usefulness of such data for typical commercial polymer surfaces is given in (14). It is also imperative, particularly for surfaces which are partially hydrophilic, which contain two or more constituents which may be distributed in block-like structures, or which contain two or more constituents one of which may be highly mobile at the interface, that the contact angles be measured under water as well as in air. In materials such as these, the surface region will tend to equilibrate with the environment in question and will appear to be hydrophobic in air and hydrophilic in water. One might conclude from a contact angle measured in air that the surface was hydrophobic when in the environment of major interest for blood-material interactions it would be highly hydrophilic. The contact angle tests performed underwater obviate these problems (10); a brief protocol is given in appendix E.

Surface Chemical Composition--X-ray Photoelectron Spectroscopy

If the surface of a polymer is considered to be limited to the topmost 100 Angstroms, the only surface analytical technique routinely available which permits one to chemically analyze a polymer surface in a relatively nondegrading and semiquantitative way is X-ray photoelectron spectroscopy (XPS), commonly called ESCA (electron spectroscopy for chemical applications).

ESCA was developed largely by the efforts of Siegbahn and his collaborators in Sweden (15). It is based on the precise measurement of the kinetic energy of electrons ejected from the sample by the action of incident radiation, usually X-rays or UV; basically, the photoelectric effect. The binding energy of the electron prior to ejection (E_B) is obtained from the measured kinetic energy (E_K):

$$E_B = E_{h\nu} - E_k - C$$

where $E_{h\nu}$ is the energy of the monoenergetic excitation radiation (Al Kα, 1487 eV in many instruments), and C is an instrument constant which is readily determined experimentally. The high precision of the E_k measurement allows one not only to identify the elements (except for hydrogen and helium) present in the surface, but also to identify their oxidation state. The photoelectron spectra can be shifted by as much as 10 eV, depending on the oxidation state of the element.

Modern ESCA instruments sample an area of the order of several square millimeters. The volume sampled depends on the photoelectron escape depth for the sample. The escape depth is of the order of 5 to 15 Å over the energy range of interest for most metals. Data for polymers and low density solids are not readily available, although the generally accepted escape depths are the order of 50 to 100 Å. Recent data using calibrated fatty acid monolyers gives a range of 35 to 70 Å as an escape depth. The sampling depth can be decreased by decreasing the angle the escaping electrons make with the sample surface. This procedure is commonly called the "glancing angle" technique.

The ESCA method thus provides a complete elemental analysis (except for hydrogen and helium) of the top 50 to 100 Å of the sample, as well as information on the bonding state of the elements present. The spectra also contain information on the molecular orbitals present and on the electrical conductivity of the surface region.

It is particulary important for all materials of interest to be examined by ESCA in a so-called wide scan (generally 0-1,000 eV), which provides a spectrum that permits one to analyze for the elements in a semiquantitative way. This is an ideal method of determining the presence of trace impurties and surface contaminants present on the surface. For example, one often finds fluorocarbon and silicone materials present on many commercial polymer surfaces due to mold-release agents; antistatic agents can also be present. Many commercially prepared polymer surfaces have a small degree of surface oxidation, which is not readily detectable by total internal relfection infrared techniques, but is readily detectable with ESCA; the degree of surface oxidation should certainly be quantitated.

After the total elemental analysis and the assurance that the surface does not contain any undesirable impurities or contaminants, it is important to analyze the ESCA spectrum at higher resolution; in the case of polymers, this means looking at the carbon, oxygen, and nitrogen regions. For example, a detailed analysis of the carbon region of the spectrum allows one to deduce the proportion of carbon atoms which are hydrophobic-type carbon (alkyl or aromatic carbons) and those which are hydrophilic (the carbon atoms bonded to electronegative or electropositive groups, i.e., polar groups) by simply comparing the ratios of the appropriate areas of the carbon ESCA spectrum. One thus obtains a direct ratio of the hydrophobic to hydrophilic character of the material.

Although ESCA instrumentation is relatively expensive, it is now available in a number of commercial laboratories and in many industrial laboratories for analytical purposes. It is a well established surface anlaytical technique and particularly useful for the characterization of polymer surfaces (16).

Protein Adsorption

The adsorption of plasma proteins is the first in a complex series of events that occurs when a synthetic material is placed in the cardiovascular system. It has been shown that adsorption can alter the enzymatic activity of thrombin and prothrombin conversion, modify the subsequent interaction of platelets and red blood cells with surfaces, and passivate the surface if serum albumin dominates the interfacial film. The detailed behavior of proteins adsorbed at the solid/solution interface is currently believed to be one of the keys both to understanding and predicting the behavior of artificial materials placed in the body. In particular, knowledge of time-dependent protein surface composition under conditions of competitive adsorption, the correlations between adsorbed protein conformation and biological properties of the protein, and the effect of the chemical nature of the surface of the material upon protein adsorption are being actively investigated.

Unfortunately, the current data on adsorption for a given protein on similar substrates shows a wide variation from investigator to investigator. This illustrates the difficulty involved in performing such measurements. The availability of well-characterized reference materials on which the adsorption of a single, well-characterized protein could be studied would remove much of the ambiguity from the results and would ensure meaningful comparisons among investigators.

Protein purity and characterization are critical aspects of these studies. It is difficult to obtain proteins absolutely free of oxidized forms, of other proteins, trace contaminants, or peptide hydrolytic products. To avoid oxidation, denaturation, and growth of bacteria, particular care must be taken in preparation and storage of protein solutions. Moreover, they should be checked for stability and purity by enzymatic, immunological or other appropriate methods.

Since many studies of protein adsorption involve only measurements of adsorbed amounts, radiotracer techniques are frequently used. Such techniques are conceptually straightforward and involve little interpretation to extract the desired information. However, possible alterations in the protein during labeling and the presence of residual label are complications which must be guarded against. A recommended procedure for carrying out these adsorption studies is the one developed at the National Bureau of Standards (17). This investigation characterized the effect of stirring, sample withdrawal, rinsing, surface roughness, and protein labeling (preferential adsorption). These factors were found to profoundly modify the adsorption properties reported and must be controlled (18). It is, in general, a good practice to vary the adsorption conditions and the ratio of solution concentration to surface area to test for complications associated with protein adsorption.

At present, human serum albumin is probably the most suitable and best characterized protein in terms of adsorption. It should be noted that albumin does have a free sulfhydryl group which is readily oxidizable as evidenced by dimerization, and that this protein is the physiologic carrier of free fatty acids and many drugs; it should be noted that even if fatty acids or drugs are present in albumin preparations, they are not detectable by protein chemistry techniques. Whether a locally or commercially labeled protein is used, it should be chromatographically purified on Sephadex G-10 prior to use. For proteins radiolabeled with iodine, the protein preparation should also be chromatographed on an ion exchange resin to remove free iodide ion. The purity of the protein and the specific radioactivities should be reported for the protein solution after any purification steps have been completed as well as at the time of each experiment. For proteins with enzymatic activity or other quantifiable functions, this specific activity should also be reported at time of use. Use of these techniques should result in reproducible results for human serum albumin adsorption. However, skilled protein chemistry techniques are required and reproducibility should be validated by studies on reference materials as well as by repeated iodinations of the protein from a single lot and from different lots.

Summary

Completion of the Level II analytical protocol (figure 13), yields a detailed measure of surface roughness and texture which is required prior to any blood-material interaction testing. It also provides a detailed measure of the surface chemistry and, as the ESCA technique probes only the top 50 to 100 Å of the polymer surface, very small levels of surface contaminants or low degrees of surface oxidation are readily detected. In addition ESCA provides a total elemental analysis of the surface further confirming the absence presence of surface contaminants. The contact angle evaluation provides a rigorous measure of the critical surface tension and, if done underwater, provides a measure of the true hydrophobicity or hydrophilicity of the material-water interface.

All the techniques recommended are readily available, readily interpretable methods, which can be carried out in a fairly straightforward manner. Although scanning electron microscopy, energy dispersive analysis of X-rays, and X-ray photoelectron spectroscopy may not be available in many research laboratories, these are methodologies which are readily available through specialty labs and through commercial analytical laboratories. Thus, level II characterization should not provide a severe burden on any researcher.

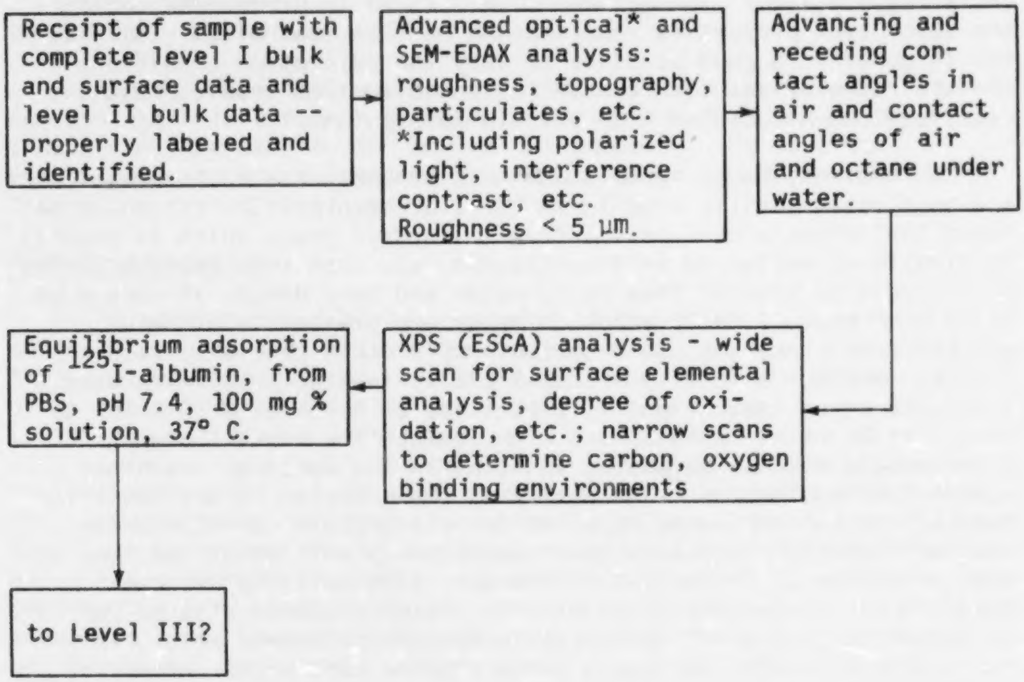
Receipt of sample with complete level I bulk and surface data and level II bulk data -- properly labeled and identified.
Advanced optical* and SEM-EDAX analysis: roughness, topography, particulates, etc. *Including polarized light, interference contrast, etc. Roughness < 5 μm.
Advancing and receding contact angles in air and contact angles of air and octane under water.
XPS (ESCA) analysis - wide scan for surface elemental analysis, degree of oxidation, etc.; narrow scans to determine carbon, oxygen binding environments
Equilibrium adsorption of 125I-albumin, from PBS, pH 7.4, 100 mg % solution, 37° C.
to Level III?

Figure 13. Flow-diagram summary of level II characterization.

LEVEL III TESTS

Surface Charge and Potential

Although there is considerable interest in surface charge and some evidence that the surface charge characteristics of a material do correlate with its blood-material interaction properties, the field is quite controversial and suffers from experimental difficulties. The techniques usually employed to measure surface charge are not applicable to a wide range of materials. Most of the standard electrochemical techniques are, of course, applicable to conductors or semiconductors. Many of the interfacial potential techniques, based on electrokinetic phenomena, are really only applicable to insulators. This is an area in which there is considerable interest and need; the working group considers it to be a problem area where more research is necessary before tests can be recommended for the routine characterization of biomaterials. A discussion of surface electrical properties is given in appendices F and G.

Measurements of surface potential difference are relatively well known and can be obtained accurately, provided samples of appropriate geometry are available. These methods are commonly used for the characterization of polymer electrets (19). Zeta potential techniques, generally based on streaming potential measurements, have been widely used for the characterization of biomaterials (20). While they are fairly straightforward, for the characterization of many types of polymers the zeta potential is a function of flow conditions and the extrapolation of the value to more realistic device conditions is difficult. The recent development of a flat plate streaming potential apparatus allows the evaluation of a reference material and other materials produced in membrane or sheet form (see appendix F), however, such an apparatus is not widely available.

Optimum surface charge characterization of conducting materials can be accomplished by standard electrochemical methods, which are discussed briefly in appendix F, and in appropriate text and laboratory books (21).

Molecular Surface Characteristics

Methods for molecular surface characterization are becoming available. Two techniques are of particular interest and will probably evolve into very useful methods for the characterization of biomaterials. Secondary ion mass spectrometry (SIMS) and laser Raman spectroscopy are both techniques in which considerable additional research work should be encouraged (22,23). Laser mass spectrometry may also be of considerable interest (24).

The sputtering of a material with 1 to 30 keV ions results in the production and release of secondary ions characteristic of the material. The escape depth of the secondary ions is of the order of

10 to 20 Å, enabling true surface analysis of the material. Since sputtering of the surface atomic layers results from the production of secondary ions, a depth profile for a number of elements can be obtained. Except for the technique of generating the ions, SIMS utilizes conventional mass spectrometry techniques and instrumentation. With appropriate optics, SIMS can be utilized as a finely focused beam (ion microscope or ion microprobe) enabling the investigation of 1 μm particles or areas.

Recent advances in the technique utilizing very low primary ion current densities have enabled the study of adsorbed organic molecules, including amino acids, peptides, drugs, and alcohols (25). The generation of parent-like secondary ions allow idenification of molecules with detection limits in the 10^{-12} g range. The technique is currently not widely in use, although a number of laboratories are expanding or initiating studies. In general, samples must be studied under high vacuum (10^{-6} to 10^{-8} torr) which limits the type of systems that can be investigated.

The laser-Raman microprobe can be employed for the characterization of molecular compounds adsorbed on both single particles and large surfaces (26,27). Detection and measurement are based on the observation of the common Raman effect. In the instrument, a beam of monochromatic, visible laser light is focused on the sample. The scattered radiation shows Raman shifted frequency differences characteristic of the sample. The Raman pattern therefore represents a molecular fingerprint for identification of surface compounds. If the sample does not absorb the incident frequency, heating can be kept to a minimum. For adsorbed organic hydrocarbons, a detection limit of 10 to 100 picograms has been established (28).

Depending on the optical properties of the sample and its reflection properties, the spectrum may originate from very deep in the sample. This is particularly true for a transparent sample. The technique may be relatively surface sensitive for an opaque or poorly scattering material. Raman spectra, of course, are of considerable interest for organic materials. It must be remembered, however, that the surface sensitivity of this technique and its routine analytical capabilities are still unproven although the technique is developing rapidly and merits careful attention.

The new laser mass spectroscopy microprobe utilizes a 1 μm laser beam which is focused through a microscope onto a particular spot on the sample. The sample is locally pyrolyzed, and the products fed into a mass spectrometer, producing a mass spectrum of the local sample region. Thus, point-by-point pyrolysis mass spectroscopy of samples can be conducted. Depending on the beam energy, each laser pulse may vaporize a volume of the order of 1 micron or more in depth. That is too deep to be considered a true surface technique, but under certain conditions it may provide surface information.

Surface Area

If a material is to be used for studies of protein adsorption and detailed blood-material interactions, it is, of course, important to obtain a measurement of total surface area. This is difficult to do for relatively low surface area materials, such as common sheets and tubes, although it may be possible by the adsorption of radiolabeled molecules from solution provided that adsorption is uniform and a monolayer is formed. A particularly useful technique has been described utilizing both ^{14}C-labelled stearic acid and hexadecane. Overnight adsorption from benzene solution followed by rinsing and counting enabled a comparison of the true surface area of platinum, quartz, and chrome materials. Similarly, Timmons et al. (29) used the technique to compare the effect of polishing iron with different size grits. A direct relationship was found between grit size and the true surface area. By using both polar and nonpolar molecules, the possibility of adsorption artifacts can often be detected. This technique can only be used for materials which do not swell in the solution and solvents of interest and for solutes which do not absorb into the material.

Moreover, it involves the assumption that the adsorbing molecules deposit in a monolayer. For surfaces with heterogeneous energy distribution, this may not be so. Therefore, there is a need for further work to assure the applicability of the method to various classes of materials. The reference materials described in chapter VI would provide a useful range of materials on which to test this method.

X-ray Photoelectron Spectroscopy - Advanced Methods

The X-ray photoelectron spectroscopy technique is also capable of providing considerable additional information. It may be possible to measure directly the number of charged groups on a surface using this method. For example, if one is interested in carboxylic acid groups, they may be quantitated by exposing the polymer to a silver nitrate, lead nitrate, or lead acetate solution, thereby forming the lead or the silver salt of the carboxylic acid. After a simple rinse and dry, the sample can be examined with ESCA and the carbon to silver ratio on the surface allows one to determine the proportion of the carbon on the surface involved in carboxylic acid groups or charged groups which bind or complex the metal cation (30). Such data using cations correlate, at least in a qualitative fashion, with streaming potential or zeta potential measurements of the charge present on polymer surfaces when the charge is due primarily to ionizable organic functional groups. In principle, a similar technique could be developed with anions, for example, iodide, to monitor positively charged groups on the surface.

It should also be noted that progress is being made in deducing electron donor-acceptor or acid-base properties from ESCA spectra as well as directly from contact angle data (31).

Summary

Total internal reflection infrared spectroscopy and, possibly, internal reflection Raman spectroscopy merit development and consideration for future surface characterization.

In the area of protein adsorption, new techniques are becoming available which may allow measurement of adsorption *in situ* and estimation of adsorbed protein conformation. These techniques include ellipsometry (32), total internal reflection fluorescence spectroscopy (33), and possibly total internal reflection ultraviolet, infrared, and Raman spectroscopy. In addition, there has been some application of circular dichroism for the detection of adsorbed protein conformation (34).

An additional major need in surface characterization is a method of determining surface morphology of block copolymer systems and related systems with microscopic heterogeneity. The standard optical and scanning electron microscopy techniques for measuring surface topography and morphology are not very useful at the 100 Å level. Nevertheless, many of the materials of interest involve block copolymers with domain morphologies ranging from tens of Angstroms to several hundred Angstroms, such as in the block copolyether urethanes.

The direct surface morphological characterization of these materials is essentially impossible at present. Currently, transmission electron microscopy of thin films is used for such studies; however, these techniques provide data more representative of bulk morphology. There is some possibility that contact angle hysteresis may give some information on micromorphological properties which result in energy microheterogeneities on the surface (35). There is also some hope that back scattered electron detection in the scanning electron microscope may help as well as ion beam etching followed by scanning electron microscopy at high resolution (36).

References

1. A.W. Neumann: "Contact Angles and Their Temperature Dependence: Thermodynamic Status, Measurements, Interpretation and Application", Adv. Colloid Interface Sci. 4, 105, 1974.
2. W.C. McCrone, J.G. Delly: The Particle Atlas, 2nd ed., Vol. Principles and Techniques, Ann Arbor Sci. Publ., 1973.
3. W.C. McCrone: "Light Microscopy," in P.F. Kane and G.B. Larrabee, eds., Characterization of Solid Surfaces, Plenum, 1974.
4. N.J. Harrick: "Transmission and Reflection Spectroscopy," in L. H. Lee, Characterization of Metal and Polymer Surfaces, Academic Press, 153, 1977.
5. N.J. Harrick: Internal Reflection Spectroscopy, Wiley, N.Y., 1967.
6. W.A. Zisman: "Relation of Equilibrium Contact Angle to Liquid and Solid Constitution," F.M. Fowkes, ed., Contact Angle, Wettability, and Adhesion, Adv. Chem. Series 43, 1, 1964.
7. J.J. Bikerman: Physical Surfaces, Academic Press, N.Y., 1970.
8. S. Hattori, R.N. King, J.D. Andrade: "Surface Tension Measurements...", J. of Bioeng. 2, 113, 1978.
9. R.E. Baier, V.L. Gott, A. Feruse, Trans. Amer. Soc. Artificial Internal Organs, 16, 50, 1970.
10. J.D. Andrade, R.N. King, D.E. Gregonis, D.L. Coleman, "Surface Characterization... Contact Angle Methods in Water," J. Polymer Sci. Symp. C., 1979, in press.
11. A. W. Adamson, Physical Chemistry of Surfaces, 3rd ed., Wiley, N.Y., 1976.
12. D.B. Holt, M.D. Muir, P.R. Grant, I.M. Boswarva, eds.: Quantitative Scanning Electron Microscopy, Academic Press, N.Y., 1974.
13. P.G.T. Howell: "Stereo Data from the SEM," Scanning Electron Microscopy/1975, 697, 1975.
14. D.W. Dwight, W.M. Riggs: "Fluoropolymer Surface Studies," J. Colloid Interface Sci. 47, 650, 1974.
15. K. Siegbahn, et al." Electron Spectroscopy for Chemical Analysis, Almqvist and Wiksells, Stockholm, 1967.
16. D.T. Clark: "ESCA Applied to Polymers," Adv. Polymer Sci., 24, 126, 1977.
17. L.E. Smith, W.H. Grant, R.E. Dehl: "Interaction of Blood Proteins with Solid Surfaces," NBSIR 77-1392, Annual Report prepared for the Biomaterials Program, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD., July 1977.
18. W.H. Grant, L.E. Smith, R.R. Stromberg, J. Biomed. Mater. Res., 8, 33, 1977.
19. J. Van Turnhout: "...Polymers for Electrets," J. Electrostatics, 1, 1975, 147.
20. R. Van Wagenen, J.D. Andrade: "Flat Plate Streaming Potential Investigations," J. Colloid Interface Sci. 1980, in press.
21. S. Srinivasan, P.N. Sawyer: "Electrochemical Techniques for Characterization of Clean Surfaces in Solution," in G. Goldfinger, ed., Clean Surfaces, Dekker, N.Y., 195, 1970.
22. J. A. McHugh in A.W. Czanderna, ed., Methods of Surface Analysis, Elsevier, N.Y., 223, 1975.

23. N.S. McIntyre, ed., Quantitative Surface Analysis ASTM STP, 1978, 643

24. M.S. Burns-Bellhorn, D.M.File: "SIMS of Standards for Analysis of Soft Biological Tissues," Anal. Biochem. 92, 213, 1979

25. A. Benninghoven, W.K. Sichtermann: "Detection, Identification, and Structural Investigation of Biologically Important Compounds by SIMS," Anal. Chem., 50, 1180, 1978.

26. R. Wechsung, F. Hillenkamp, R. Kaufman, R. Nitsche, H. Vogt, "LAMMA-A New Laser Microprobe Mass Analyzer," Scanning Electron Microscopy/1978, Vol. 1, 611, 1978.

27. J.F. Eloy: Microscopica Acta, Suppl. 2, 307, 1978.

28. G.J. Rosasco, E.S. Etz: "The Raman Microprobe," Res/Dev., 28, 1977.

29. C.O. Timmons, R.L. Patterson, L.B. Lockhart,Jr.: "The Adsorption of C14-Labeled Stearic Acid on Iron," J. Colloid Interface Sci., 26, 120, 1968.

30. J.S. Hammond, J.W.Holubka, R.A. Dickie: Organic Coatings and Plastics Preprints, ACS, 39, 506, 1978.

31. F.M. Fowkes and S. Maruchi: Organic Coatings and Plastics Preprints, ACS, 37, 605, 1977.

32. B. Morrissey, L. Smith, C. Fenstermaker, R. Stromberg, W. Grant, "Conformation of Adsorbed Blood Proteins," in Nat. Bur. Std., Public. 415, Biomaterials, 83, 1975.

33. R.W. Watkins and C.R. Robertson: "Total Internal Reflection Technique for ... Protein Adsorption," J. Biomed. Materials Res., 11, 915, 1977.

34. C.R. McMillin and A.G. Walton: "A CD Technique for the Study of Adsorbed Protein," J. Colloid Interface Sci., 48, 345, 1974.

35. R.E. Johnson, Jr., R.H. Dettre: "Wettability and Contact Angles," in E. Matijevic, ed., Surface and Colloid Science, Vol. 2, 85, 1969.

36. D.A. Thomas, "Morphology Characterization of Multiphase Polymers...," J. Polymer Sci., Symp. 60, 189, 1977.

CHAPTER V

BIOLOGICAL TESTING

TOXICITY EVALUATION

Introduction

The ultimate safety and performance of materials used in cardiovascular applications depend simultaneously upon satisfactory physical performance and compatibility with the biological system under conditions of end use. Toxicological testing provides information on the potential toxicity of materials in the final clinical application, on the presence of leachable constitutents in the material, and on the possibility of other biological effects which may translate into undesirable consequences in the final device. Typically, this testing has been carried out under contract with laboratories that specialize in these procedures.

The toxicological testing of materials has unique constraints which limit the applicability of classical protocols and procedures. Selected reviews of the toxicology of plastics and the testing of materials are listed in references (1-6). The fundamental problem in the application of classical protocols, developed largely in the area of drug development and evaluation, to a synthetic material, is that even the most homogeneous, is a complex entity, whose toxicity is mediated by both its physical and chemical properties. Classical protocols are almost exclusively designed to obtain quantitative dose-response data on pure chemical agents, e.g., drugs or toxicants. In the case of synthetic materials, chemical toxicity derives from leachable toxic components of the material; in contrast to drugs or food additives, the complete chemical composition of a synthetic material is often not known. Even if the chemical composition of a material is completely known, good toxicological information is seldom available on the components, which frequently have never been tested. Possible synergistic reactions among the components in any given biological test system are seldom known. This makes the application of classical protocols, beyond the exercise of good experimental design and rigorous application of the scientific method, essentially inappropriate for the testing of complex substances such as biomaterials.

The toxicity testing of materials is directed toward engineering goals; that is, toward the assessment of the potential toxic liability of the material under specific conditions particularly under "end use" conditions. The approach to such evaluation is, therefore, highly empirical. It is in the evaluation of the empirical toxicity data that the large body of fundamental information derived from classical studies comes to bear on the assessment of materials. The relative significance of toxicological responses (acute, subacute and chronic) to the health of patients or the "safety" of chemical agents has been broadly established for diverse drugs and chemical agents, and it is in that context that the empirical toxicological data generated on materials used in cardiovascular applications must be assessed.

As in the area of drug evaluation, the clinical trial is the most pertinent, although the most difficult and costly, test of the safety and efficacy of a material or a biomedical device. The rigorous application of the fundamental concepts of toxicology in preclinical evaluation of such materials or devices to ensure ultimate safety under end use conditions is the challenge faced at all levels of biomaterials testing.

Toxicity testing should take place at logical points in the development of materials and devices. The juncture at which the tests are performed dictates the type of test(s) appropriate. Two logical junctures for toxicity screening are: 1) early in the synthesis program, as soon as a material is identified as a candidate material; and 2) as soon as the "end use" configuration of the material is determined and the "device" (or component of a device) is available for testing. Certain tests are appropriate for both situations; however, "end use" testing will carry certain constraints unique to the final configuration and context of application of the device. The appropriate junctures for testing between these two points in time are determined by the variables introduced in the design and fabrication process (recognizing that both chemical and physical factors are involved in the manifestation of toxicity), the availability of appropriate quantities and configurations of materials/devices for testing, and judgment with respect to the need for testing at a given point. That is, if the next phase of development is time consuming and costly, it is prudent to assess the toxicity of the materials or components entering that phase.

Acute toxic responses are closely related to the kind and quantity of leachable chemical substances in the materials or devices. While, as a general principle, the absence of such substances is desirable, most materials, when subjected to rigorous extraction procedures, will yield detectable quantities of chemical substances when the extracts are examined by sensitive physical or chemical techniques. It is important, therefore, that an assessment of the potential toxic liability of the material be obtained to guide the synthesis and development program (to preclude the rejection of a material based on chemical analysis alone) and to alert the developers to potential toxic liability of apparently "minor" or undetected substances. The manifest toxicity of the material is a crucial consideration since toxicity of complex mixtures cannot be quantitatively predicted on the basis of known chemical composition. Chemical analysis in conjunction with toxicity testing can frequently identify problems at an early juncture and prevent costly developmental work on unsatisfactory materials.

Tests for leachable toxic substances are, therefore, pertinent at every major juncture in the development of a material or device. The appropriate conditions for the tests should approach "end use" conditions as the material or device approaches "end use" configuration. Tests can be performed directly on the material and/or on appropriate extracts of the material. In this connection, however, it should be noted that materials are frequently formulated with coatings of various kinds and it is the response of the coated surface that is of concern. This may dictate the use of particular tests and test conditions.

"Subacute" toxicity testing should begin as soon as the final projected formulation of a material or component of a device is known. When a material or component of a device is expected to enter the final device configuration without significant modification, "chronic" toxicity testing should be initiated to avoid long delays after final formulation of the device. Because of the cost and time required for such testing, careful consideration should be given to the most appropriate time and device configuration for testing. In addition, to the extent possible with analytical chemical techniques, the potential extractables should have been identified as to type and level, and reproducibility of material from lot to lot should have been demonstrated.

Leachable substances may pose a significant problem with respect to aspects of biocompatibility other than acute toxicity and their presence places greater demands on evaluation at the subacute and chronic levels of testing if the material is to be considered as a serious candidate for use in a cardiovascular device. Therefore, these toxicity tests in no way replace that need for extensive blood-material interaction testing.

The following sections describe tests appropriate for the assessment of acute, subacute and chronic toxicity. The tests described have been selected for broad applicability to diverse materials and for relative simplicity and ease of execution. However, other tests may be equally pertinent or superior to the proposed tests in specific situations. In any case, judgment is required both in the selection of tests and assessment of test results.

Since the principal acute toxic liability of materials is associated with the presence and leachability of toxic substances or components of the materials, the principal focus in these tests is on the detection of such substances. Chemical analysis per se of materials or extracts of materials is useful but limited in value with respect to the assessment of the potential toxic liability of detected components for two reasons: biological response is often manifested at concentrations below the limits of detection of routine analytical procedures and the potential toxic liability of detected substances (frequently complex mixtures of substances) is extremely difficult to assess. Thus, direct assessment of the toxicity of extracts currently provides the best strategy for the evaluation of toxic liability. However, this does not preclude the need for, and value of, assessing the material toxicity directly as well.

In keeping with the concept of levels of testing introduced in earlier chapters, level I tests refer to those "quality control" tests that would be useful and appropriate at frequent intervals in the evolution of materials from raw form to final device. Level II tests represent a prudent, broad assessment for new or previously untested materials. These tests constitute what is referred to as a "primary screen" which is highly useful in comparing the material with others to determine its relative merits, and in establishing a baseline against which to judge changes that occur during fabrication and development. Detailed protocols for each of these tests are available (7).

LEVEL I TESTS

These tests comprise a limited series of the more sensitive measures of toxicity. These tests assess the ability of extractable components to inhibit cell growth, to cause cell death, or to provoke an inflammatory response. While these tests are helpful in screening materials, they are also useful for maintenance of quality control during production or fabrication of a material previously tested.

Agar Overlay: Material

Leachable toxic components of materials are detected by observation of the response of a mouse fibroblast monolayer separated from the test material by a thin layer of agar. The magnitude of the response zone and the severity of the response (degree of lysis of the cells) are recorded. Any solid material, e.g., bulk solids, sheets, films, powders or fibers, which can be placed directly on the agar surface of the test plate (approximately 1 cm^2 in contact with the agar) can be tested; extracts of materials may be applied to cellulose disks which are placed on the surface in the same manner (see below). Clean but not necessarily sterile samples are required and positive and negative control materials must be available.

A monolayer of mouse fibroblast cells is propagated on the bottom of the petri dish, the growth medium is removed and the monolayer is overlaid with a thin layer of agar containing minimum concentration of supporting nutrients and the vital stain, neutral red. Duplicate test material samples and positive and negative controls are placed symmetrically on the surface of the agar, two plates per test. The plates are evaluated after 24 hours incubation, 37° C. Cell death due to toxic substances diffusing from the sample causes loss of neutral red dye from the cells; the test is considered valid if the positive and negative control samples show the anticipated standard response. The response is evaluated grossly and microscopically and graded in terms of the size of the region of dye loss (zone index) and the percent of the cells lysed within the zone regardless of zone size (lysis index) (table 2). The test results are reported in terms of a response index (RI), defined as zone index--lysis index, which varies from 0/0 (no detectable response) to 5/5 (zone covering entire plate and over 80 percent of cells lysed).

TABLE 2. Agar Overlay Tissue Culture Test

Zone Index:	Zone of vital dye loss
0	No detectable zone around or under sample.
1	Zone limited to area under sample.
2	Zone not greater than 0.5 cm in extension from sample.
3	Zone not greater than 1.0 cm in extension from sample.
4	Zone greater than 1.0 cm in extension from sample but not involving entire plate.
5	Zone involving entire plate.

Lysis Index:	Degree of cell lysis (microscopic)
0	No observable lysis.
1	Up to 20 percent of the zone lysed.
2	20 to 40 percent of the zone lysed.
3	40 to 60 percent of the zone lysed.
4	60 to 80 percent of the zone lysed.
5	Over 80 percent lysed within the zone.

Positive and Negative Controls. A suitable positive control material will routinely produce a standard response index in repeated tests. The magnitude of the RI should be in the range of 2/3 to 3/4 but should not vary with respect to time. Poly(vinyl chloride) sheets formulated with four parts Thermolite (organotin) has been found to be satisfactory and stable for several years.

Any material which will routinely produce a response index of 0/0 is suitable for a negative control. High purity polyethylene sheet material has been found to be satisfactory.

Agar Overlay: Material Extracts

The agar overlay tissue culture test can also be used to evaluate the toxicity of extracts of diverse solid materials obtained under selected extraction conditions. Such a modification had the advantage of being independent of the form of the solid material tested, and of identifying clearly that the toxicity is related to a leachable component. A number of standard extraction media and conditions of extraction have been extensively employed in primary acute toxicity screening of materials. These media and conditions avoid the inherent stability problems of the complex physiological solutions they are designed to mimic. The extraction media used include distilled water, saline, polyethylene glycol-400 (PEG-400), and cottonseed oil. Water and saline are particularly useful because the aqueous extracts can be combined with other additives to make up a variety of "physiological" solutions. PEG-400 is moderately nonpolar, and extracts must be diluted 1:8.3 (extract:saline) by volume, since undiluted PEG-400 is, itself, toxic. Cottonseed oil is highly nonpolar and extracts the more nonpolar leachables which are often the more toxic. It is advisable, however, to ensure that the oil batch is nontoxic in a control test. For level I testing, three different solvents should be employed.

The extraction protocols are similar to those used for testing plastic containers (USP XIX). Standard quantities of materials, based on surface area or weight, are cut to appropriate dimensions and extracted in a sealed tube with the medium of choice for a specified time and at selected temperatures. Two temperatures are recommended: 121° C (autoclave) and 37° C (incubator or water bath). The higher temperature affords more rigorous extraction conditions and is recommended for initial acute toxicity testing. Negative results with such extracts provide evidence for the chemical stability of the test material and the absence of substances soluble in the extraction medium. If extracts obtained at high temperature are toxic, extraction at 37° C should be performed to determine whether thermal degradation products may have contributed to the observed toxicity.

The duration of extraction is important with respect to assessment of leachability. The high temperature (121° C) extraction is usually carried out for 1 hour, following the recommendations in the USP, permitting the evaluation of extracts on the same day as the extraction. Extraction at 37° C is usually carried out for longer periods of time, e.g., 3 days. Under these conditions, an antibiotic, e.g., 100 μg/ml of gentamycin, can be added to aqueons extraction media.

Extracts are imbibed into cellulose assay disks (0.1 ml per disk) and applied to the agar as described above.

Intradermal Irritation Test

The intradermal irritation test is an even more sensitive test of acute toxicity; the results of this test, however, correlate with the results of *in vitro* tests. For this test, extracts of materials, or solutions of suspected leachables may be employed. Sterile extracts are desirable but not required and a small animal facility for the handling and holding of rabbits is necessary. The test is particularly useful in detection of leachable, nonpolar components of materials and takes about 4-5 days. Irritancy, although correlated with acute toxic response, is a qualitatively different response than that assessed in the other acute toxicity tests described and is an important parameter in the evaluation of materials.

Extracts of materials obtained with nonirritating extraction media are suitable for the test. Saline, PEG-400 and cottonseed oil are routinely employed. The extraction medium is employed as a negative control and 20 percent ethanol in normal saline is a positive control. The extraction method is as described in the preceding section and the test procedure is described in USP XIX. It involves the intracutaneous injection of the extract into rabbits and the assessment of irritant response, with or without the use of vital dyes. The dyes have the advantage that irritant responses can be observed very rapidly, often within the first hour. The standard test without dye requires 72 hours.

Inhibition of Cell Growth

Toxicity may also be observed as inhibition of cell growth under defined conditions. This is one of the more informative tests for leachable substances and is more sensitive than the short-term acute tissue culture tests described above, but does require more time and technical skill.

Distilled water extracts are prepared as described above in a series of graded weights of material (six weights ranging from 4,000 mg to 1 mg/20 ml). The extracts are incorporated into tissue culture medium and inoculated with a standard quantity of mouse fibroblast cells in tissue culture assay tubes. The tubes are incubated for 72 hours, the medium is decanted, the cell monolayer is gently washed with buffered saline to remove unattached cells, and the extent of cell growth is determined by total protein assay on the individual tubes less the amount of protein in the initial inoculum (zero time). The difference in growth in the presence and absence (negative control) of the extract is expressed as the inhibition of cell growth (ICG). The results are reported for each weight of material extracted and presented as a plot of percent inhibition against weight of material extracted.

LEVEL II

The level II toxicity tests comprise a set of broadly applicable and relatively simple tests which should be used for the initial evaluation of biological compatibility of a new material before costly development work is undertaken. The set of acute toxic response tests includes *in vitro* and *in vivo* tests of a material or extract and is referred to as the primary screen. To provide a basis for comparison of results a cumulative toxicity index (CTI) has been devised; the CTI is the sum of scores from the primary screen.

Materials that have favorably passed the primary screen, have been assessed for their interactions with blood and whose physicochemical or mechanical properties are known should be further evaluated in subacute and chronic toxicity tests. These *in vivo* tests are time consuming and costly and so are appropriately applied to materials in an advanced stage of development.

As with other types of studies, positive and negative control materials (reference materials) are necessary to calibrate the results.

Tissue Culture: Materials

The tissue culture test is similar to the agar overlay test except that test material is placed directly on top of a monolayer of cultured cells without an intervening agar layer, and culture medium surrounds the test material. Thus, the potential "barrier effect" of an interposed agar layer is avoided and the extraction or leaching of components from the material takes place under conditions more closely approaching "physiological."

The test material is formed to provide a flat disk, approximately 1 cm in diameter, which can be placed on the cell monolayer. If the sample is too light to remain firmly in place in the liquid medium, it can be weighted with sterile glass weights. Fibers, powders, gels or loose-textured materials are not usually suitable. Samples must be sterile. If chemical sterilization is employed, extreme care must be exercised to ensure that the sterilant is completely removed before testing.

Several versions of this test have been developed. Usually the procedures involve the following basic steps: a) the propagation of a monolayer of mammalian cells on the bottom of a petri dish in liquid tissue culture medium; b) the placement of a solid test sample on the monolayer directly in the liquid medium in the petri dish; c) removal of the test sample and liquid medium after a standard incubation period (usually 24 hours or more); d) staining of the cell monolayer, e.g., with trypan blue, to identify the zone of dead cells; e) the measurement of the area of dead cells (cell counts are sometimes used); and f) reporting of a toxicity rating in terms of the area of dead cells as compared to the areas observed with the positive and negative controls.

Cell Growth on Test Materials

The ability of cells to grow at or on solid surfaces, with or without adhesion to the surfaces, is an important aspect of the functional properties of materials used in implantable devices. Although *in vivo* assessment of this parameter is essential, preliminary assessment of cell growth on solid surfaces can be made *in vitro*. Various tests with many different cell lines have been devised.

The growth characteristics of mammalian cell lines propagated in contact with the solid surface of the test material are assessed in terms of the rate of growth, the ability to form monolayers of cells on the surface, the morphology of the cells and the degree of adhesion of the cells to the test surface. Growth characteristics are correlated with known physicochemical properties of the solid surface. This test is necessarily affected by the type and quantity of leachable substances in the test material; however, the test is considerably more subtle than those directed toward the assessment of acute toxicity.

The test samples can range from smooth to textured surfaces, either flat or in specific geometric configuration, limited only by the need to introduce the test sample into a liquid tissue culture medium. The test samples must be sterile and free of any chemical sterilants.

Although test protocols are not yet standardized, the basic procedure involves a) the preparation of a test material with the surface properties to be tested; b) the introduction of the test material into an appropriate liquid tissue culture system; c) the addition of a suspension of the selected cell line; d) the incubation of the cells in contact with the test surface and e) assessment of the growth characteristics of the cells after specified periods of incubation. After incubation, the numbers of cells attached to the surface, their morphology and their ability to propagate as a mono- or multilayer is assessed. If the surface is transparent, conventional microscopic techniques may be used; otherwise indirect assessments are required and may lead to artifact. Growth characteristics (number, morphology) are compared to that observed on reference materials.

Hemolysis

Short-term acute hemolytic activity is significant with respect to potential acute toxic liability of biomaterials. In general, the concentration of a toxic or hemolytic substance which produces overt hemolysis is approximately an order of magnitude higher than that required to inhibit cell growth in tissue culture and is correlated with acute toxicity in vivo. The detection of overt hemolysis is, therefore, of immediate significance with respect to toxicity. The test is rapid, requires simple equipment and can be performed in the presence of the material or on the "extract" after removing the material to assess indirectly the contribution of the physical surface of the material to manifest hemolytic activity.

Test samples are cut such that 5 gm of material can be submerged in 10 ml of saline. Where this is not possible, the maximum amount of material is added which still allows blood to be freely mixed with the suspension. Clean but not necessarily sterile samples are required.

The test consists of allowing the test material to equilibrate with saline at 37° C for 30 minutes, addition of 0.2 ml of a standardized rabbit blood with gentle mixing, incubation for 60 minutes at 37° C, centrifugation of the suspension and spectrophotometric determination of the hemoglobin content of the supernatant saline solution. The results are compared to a totally hemolyzed sample less the amount of hemolysis induced by a negative control (percent hemolysis).

Intramuscular Implants

Intramuscular implantation of materials in rabbits provides a direct assessment of acute tissue reaction to materials and can be performed with simple equipment. The acute test execution takes about 10 days to complete and it is considered one of the more pertinent and direct tests for leachable components of materials. It is useful in that it provides an assessment of tissue reaction to materials under physiological conditions. Leachable components diffuse radially from the implant and the extent and severity of the tissue reaction is measured. Any solid material which can be cut or formed into small rods of approximately 0.5 to 1.0 mm diameter by 1 cm long is suitable as a sample. Aggregates of fibers or powders can be implanted but abnormal responses are frequently observed with such samples. The rods should be clean but not necessarily sterile. The samples are implanted using a trocar needle into the paravertebral muscle of rabbits along with positive and negative control implants. For acute studies (primary screen) the rabbits are sacrificed after 7 days, the implant sites with their adjacent tissue are excised, the gross evaluation of tissue response is recorded, and the implant site is preserved in formalin for histopathological evaluation of tissue response.

It is possible, however, that time-dependent release of toxic leachable components may extend over longer periods and, in cases where the material degrades in vivo, delayed responses may occur. For these reasons, subacute and chronic extensions of the short-term test are useful.

The subacute test is conducted for 12 weeks using a sufficient number of rabbits to allow sacrifice and evaluation at weekly intervals. It is designed to determine time dependency of recovery from any observed acute response and to detect delayed response over a moderate period of time. A material showing a positive response in this test, particularly a delayed or protracted response is probably not a good candidate for long-term implantation.

Chronic intramuscular implantation tests are conducted for periods of 2 years or more and may involve animals other than rabbits. These extensive tests are appropriate for materials which are strong candidates for use in devices by virtue of their physical and biological properties. The tests are designed to provide a preclinical evaluation of any histopathological problems. In view of the variety of possible end uses of the material and differences in the size and form of any device in which it is incorporated, the specific animal system, implant location, and experimental protocol must be chosen specifically to fit each particular situation. Every attempt should be made to ensure that the test conditions approximate the end use conditions.

Tissue Culture: Extracts

Extracts of materials may be included in tissue culture medium to assess toxicity to monolayers of cells. Since the extract is added to the growth medium, the extracting medium must be compatible and therefore is usually physiological; serum can be employed. Because physiological media are employed, long extraction times at body temperature are required; therefore it is necessary to have sterile samples, and to use aseptic extraction procedures.

Extractions can be performed with tissue culture medium, serum, basal tissue culture medium without serum, distilled water or, indeed, any version of the extraction medium which can be adjusted to the final standard composition of the tissue culture medium to be used in the assay using the extraction procedures described earlier. A predetermined number of cells is added to the petri dish containing the extract and to standard tissue culture medium. The cultures are incubated for a fixed period, usually 24 hours, the medium is decanted, the "monolayers" are washed free of unattached cells, the cells are stained to reveal dead cells, and the number of viable cells are estimated by direct counts. The degree of inhibition is expressed as the difference in cell counts between the treated and control sets compared to the control set alone.

Erythrocyte Stability (Osmotic Fragility)

This test provides a very sensitive measure of the interaction of leachable substances with the plasma membrane of erythrocytes, reflected as changes in the osmotic fragility of the erythrocytes on short-term exposure of the erythrocytes to material extracts. It can detect leachable components of materials at concentrations one to four orders of magnitude lower than those detectable by tissue culture tests although the major response occurs at or slightly below, the concentration range for which tissue culture systems are sensitive. The quantitation of the fragility response is also more precise and reproducible than that for most tissue culture systems. It is reasonable to assume that leachable substances which interact with the erythrocyte membrane would also interact with other biological membranes.

Any extract which is optically clear and free of particulate matter can be tested with appropriate controls, if it can be adjusted to an osmolarity in the required hypotonic range. Hypotonic saline or distilled water which can be adjusted to the repaired tonicity are most suitable. Since these extracting media are stable and do not support bacterial growth, a wide range of extraction conditions can be employed. Extracts are adjusted with distilled water or NaCl to form a solution of osmolarity appropriate to hemolyze about 50 percent of the erythrocytes on the basis of hypotonicity alone. Rabbit blood is usually used, diluted appropriately with isotonic saline (about 1 percent hematocrit). One-tenth of an ml of this stock erythrocyte suspension is added to 5 ml of extract or a control hypotonic solution and the surviving cells are counted. The relative hemolysis (number of cells lysed in the extract/number of cells lysed in the control) is reported. It should be noted that greater or less hemolysis with the extract (as compared to the control) indicates membrane interactions. By performing the test with a series of dilutions of the extract, the concentration of extract at which no detectable change occurs can be established and compared with data from other materials or from extracts prepared under different conditions.

Cell size distribution profiles can also be obtained in this test, giving an indication of the degree of swelling or morphologic changes induced in the erythrocyte population. Significant shifts in cell size distribution can be detected at extract concentrations one to two orders of magnitude lower than that producing measurable osmotic fragility changes.

Isolated Rabbit Heart

This test is fairly elaborate and technically demanding but is particularly appropriate for the assessment of the potential cardiotoxic activity of leachable components of devices that interface with the circulatory system. The test is based on the direct measurement of changes in the force of contraction of the perfused isolated heart with respect to time. The force of contraction during initial perfusion with physiological solution serves as the negative control, and changes in the force of contraction during subsequent perfusion with extract-treated physiological solution, expressed as a percent of control, constitutes the inhibition. Distilled water extracts of materials are used to formulate the test solution--approximately 1 liter of Chenoweth's solution. Normal Chenoweth's solution made up with distilled water serves as the control. In the test, the isolated rabbit heart is mounted in an Anderson Coronary Perfusion Apparatus with the heart attached to a force displacement transducer, the force and rate of contraction being recorded on a polygraph. Perfusion with normal Chenoweth's solution is initiated and continued until the force and rate of contraction has stabilized (approximately 30 minutes). Perfusion with the Chenoweth's solution containing the extract is then initiated and the force of contraction is recorded for 60 minutes. Changes in the force of contraction are measured continuously and recorded at 5-minute intervals. The test is usually run on five hearts at each of several different extract concentrations, yielding data on contraction change versus time for each extraction concentration and change in contraction with respect to extract concentration at each time.

Systemic Toxicity

This involves the simple direct intravenous or intraperitoneal test of extracts of materials in mice. It shows a low frequency of positive responses but positive responses have been observed in the absence of high positive responses in other acute toxicity tests. Moreover, this is one of the few systemic response tests and provides a more direct assessment of neurotoxicity or other adverse systemic effects than do other tests. It takes about 8 days to perform. Saline, polyethylene glycol-400 and cottonseed oil extracts of materials are usually employed and the extraction medium itself serves as a negative control. Details of the method are given in USP XIX.

In brief, mice are injected i.v. (saline) or i.p. (cottonseed oil or PEG) at a dose level of 50 ml/kg. They are observed over a period of 1 week and deaths or signs of toxicity are recorded.

In Vitro Mutagenicity (Modified Ames Test)

Since a relationship is recognized between mutagenicity and carcinogenicity of chemical agents, an assessment of the potential mutagenicity of leachable components is useful in the evaluation of materials and devices. This test provides such an assessment. Histidine auxotrophs of *Salmonella typhimurium* will not grow in minimal medium lacking histidine. Chemical agents, which cause a back mutation (or reversion) of the bacterium to a genetically competent state where the essential amino acid, histidine, is synthesized by the cell, allow the cell to grow on such minimal medium; such back mutations can be detected by the observation of growth of discrete colonies of cells deriving from the primary revertant cell. The application of extracts imbibed in assay disks to the test plate, permits radial diffusion of the soluble components of the extract into the agar seeded with the bacteria cells revertant colonies appear as a radially symmetric zone of growth.

The test takes approximately 3 days to conduct and requires standard bacteriological facilities. Sterile extracts are required and nontoxic extraction media such as distilled water, saline, or dimethyl sulfoxide are routinely used. The extract is imbibed in a cellulose assay disk and placed on the surface of the agar of the test plate. Solid samples can also be tested directly by placing a disk (approximately 1 cm^2) of the material directly on the plate. Testing of both solid and extract are recommended. Several strains of Salmonella typhimurium have been developed for this test. Testing with a minimum of two or three strains is recommended. The protocol follows the qualitative Ames test procedure and involves the preparation of agar plates with minimal medium seeded with a standard number of cells. The test sample is placed on the surface of the agar, incubated at 34° C for 48 hours and the presence or absence of revertant colonies in the diffusion zone is recorded. Parallel negative control plates (extraction medium) and positive control plates (known mutagen) are included. A description of the size and visual characteristics of the revertant colony zone is reported, along with the size of zones of toxicity which may be present and manifest as symmetrical regions of no growth around the sample.

Gap Areas

Mutagenicity, Teratogenicity, and Carcinogenicity Testing

Mutagenicity of a material is defined for present purposes as the potential of the material to cause mutations in the germ cells of an animal, thus affecting its offspring. Teratogenicity is its potential to effect alterations in the fetus during development. Finally, carcinogenicity is its potential to induce a cellular mutation leading to the production of malignancies. The three phenomena appear to be related and the assessment of the properties of candidate materials with respect to each of them is clearly important.

Classical protocols exist for assessing the mutagenicity of drugs in rats, but the application of these techniques to materials has not been validated. Teratogenicity protocols involving female rats also exist, but their validity is not even well-established for known chemical agents.

The assessment of the potential carcinogenicity of chemical agents per se is an extremely complex problem requiring large numbers of animals, rigorous environmental control, and detailed biological observations. The evaluation of solid materials is all the more difficult because of the added physical and mechanical phenomena which many believe to have a role in the induction of cancer.

None of these protocols can be considered well-established and all represent areas in which further work is needed to yield reliable quantitative data.

Reference Materials for Biological Testing

As in all aspects of materials testing, biological tests require controls to establish a quantitative scale for scoring responses and to allow the intercalibration of tests conducted in different laboratories. Polyethylene is often used as a negative control in biological tests, but materials displaying graded levels of positive response are not presently available. Further work is necessary to determine appropriate candidate materials for this purpose and to standardize the response of those materials to the toxicity test protocols.

STERILITY TESTING

Sterilization

Sterilization is a necessary and important part of the manufacture of any cardiovascular device. It must also be integrated into the materials and device development and characterization procedures for two reasons. First, the sterilization process can have a significant effect on the physical, chemical, and toxicological properties of the device and its materials. Second, the device or material, depending on its properties and geometry may actually protect bacteria or their spores from certain sterilization processes. This is especially true of porous and crystalline materials. Therefore, following sterilization it is important to assure that the materials properties have not changed as a result of the process and to assure that the device is, indeed, sterile. The level I testing procedures described in this report will accomplish the necessary physicochemical and toxicological characterization of the device. Guidelines for validation of sterility have been thoroughly addressed by other organizations within government and industry and, therefore, only a brief overview of the issues and approaches in sterilization and sterilization assurance is presented in the following paragraphs.

Sterility as defined is an absolute condition of complete destruction or removal of all forms of life; however, because of the practical difficulties involved even in the measurement of sterility (9), the best that can be stated about a sterilization process is that it reduces the probability of survival of a viable organism in the sterilized object to an acceptably small value. In general,the acceptable probability of microbial contamination of a sterile object is 10^{-6} or less, although there is presently some debate on varying the probability level in accordance with product end-use.

Methods of sterilization are classified as either physical or chemical. Physical methods include heat and ionizing radiation. Chemical methods utilize gases and liquids. Most liquids are used as disinfectants (they do not kill spores) rather than sterilants with two notable exceptions, formaldehyde and iodine (especially in combination with anionic detergents). However, liquids should not be used for microporous materials or devices with a complicated geometry since trapped air is difficult to eliminate and acts to protect microbes from the solution. Liquids should only be considered as a last choice and will not be discussed further.

Dry heat sterilization is not considered useful for most polymer materials since temperatures in excess of 140° C are required for long periods of time. Glass and metals can be processed by this method with the recommended times and temperatures listed in table 3 (10). Most plastics soften or deform

TABLE 3. Sterilization Conditions for Biomaterials

Methods	Sterilization Conditions	Comments
Dry Heat	170° C - 60 minutes 160° C - 120 Minutes 150° C - 150 minutes 140° C - 180 minutes	Can be used for glass, metal, silicon, nylons, PTFE. Longer times above 170° C can effectively inactivate endotoxins.
Steam under Pressure	121° C (15 psig) - 15 min. 126° C (20 psig) - 10 min. 134° C (29.5 psig) - 3 min.	Generally suitable for plastics. Softening and distortion may occur.
Ionizing Radiation	2.5 M rad	Embrittlement may occur. Can affect PP, PMMA, and PTFE.
Ethylene Oxide	Relative humidity 40 percent; 55° C	Requires degassing for 7 days at room temperature before use.

at these temperatures or are oxidized. Some commonly used medical materials that can be sterilized by dry heat are PTFE and silicon rubber (11). The advantage of dry heat sterilization, especially for glass or metal, is that endotoxin (pyrogen) is inactivated at temperatures of 180° C for 4 hours (12). The subject of pyrogen removal will be discussed later.

The disadvantages of dry heat are discussed in detail by Ernst (11). Briefly they are: 1) diffusion and penetration of heat is slow; 2) it requires long sterilizing times; 3) high temperatures are required; 4) many materials are damaged by heat or oxidation; and 5) temperature zones tend to develop and must be overcome.

Moist heat sterilization (autoclaving) is at least seven times more effective at killing spores than dry heat at the same temperature because of its latent heat of vaporization (10). The U.S.P. defines steam sterilization as heating in an autoclave with steam-saturated air under pressure (15 psig) for at least 15 minutes at a minimum temperature of 121° C. The 15 minute time period should not start until the material to be sterilized reaches 121° C (8). Shorter times can be used with increased temperature (see table 3).

Many polymers are unsuitable for autoclaving because of heat distortion, loss of mechanical properties and loss of physical properties. This is especially true if the material is subjected to repeated autoclaving. Rubbers can lose their properties on cooling and crystalline polymers or polymers with crystalline regions may be drastically changed after autoclaving (14). Table 4 attempts to list some common polymers and the effects of sterilization. If one is uncertain about the thermal stability of a particular polymer, the information is available in the "Modern Plastics Encyclopedia" (15).

Ionizing radiation is frequently used for catheters, some sutures, and tubing (14). Most plastics are affected in some way by radiation but dose levels employed in sterilization procedures (2.5 megarads) are usually low enough to avoid any significant changes in most polymers (see table 3). Oxidation can occur with rubbers and some polymers become brittle (14). Radiation is especially hard on PTFE, PMMA, and PP (11).

Gas sterilization can be extremely effective if certain conditions are met. Although many gases are reportedly germicidal (ethylene oxide, formaldehyde, propylene oxide, beta-propiolactone, ozone, methyl bromide, and others) only ethylene oxide and formaldehyde are considered to be useful sterilants (16).

Formaldehyde gas prepared from paraformaldehyde is reported to be one of the best available methods for sterilization (16). However, it is also reported to be ineffective for porous materials since the gas tends to condense on certain areas (17). Formaldehyde

TABLE 4. Suggested Sterilization Methods for Polymers *

Polymers	Comments
Polymethylmethacrylate	Heating above 100° C will cause distortion. Ionization will cause discoloration, cracking, and embrittlement. Ethylene oxide (EO) is good, but will not penetrate bulk material--not good for some molded devices.
Polystyrene	Polymethylmethacrylate comments apply.
Polyvinyl alcohol	Heat below 120° C can be used. EO can be used with adequate degassing. Iodine and formaldehyde will react.
Polyvinyl chloride	Autoclaving can be used if carefully packed to avoid distortion. Radiation may liberate HCl and cause discoloration. EO is good with proper degassing.
Polyurethanes	Autoclaving, radiation, and EO are all good.
Nylon	Autoclaving can cause changes in physical properties. Dry heat can be used. Water uptake may reduce the effectiveness of EO. Radiation can be used. Avoid formaldehyde.
Polyester	EO and autoclaving are excellent. Radiation may cause discoloration.
Polyethylene	Avoid heat unless material is high density. May become brittle with radiation. EO can be used.
PTFE	Heat can be used safely.
Silicones	Heat can be used safely.
Polyvinyl pyrrolidone	Autoclave at 121° C.
Natural rubber	Heat is good. Avoid radiation.
Cellulose acetate	Softened by heat. EO is satisfactory. Radiation causes loss of strength.
Regenerated cellulose	May be autoclaved. Radiation may reduce strength.

* Modified from reference (14).

is quite reactive with poly(vinyl alcohol), collagen and gelatin (14).

Ethylene oxide used as a sterilant requires an adequate understanding of the parameters affecting its activity. Its potential usefulness has been hampered somewhat by misinformation and improper use of this gas (18). It is highly explosive in air in its pure form, but the addition of carbon dioxide or freon reduces this risk. The inhalation toxicity is reported to be mild and nonaccumulative. The maximum safe level in an implanted device is 250 ppm. Bruck has reported that ethylene oxide should not be used to sterilize heparinized surfaces because the gas appears to displace the ionically bonded heparin (17).

The disadvantages of ethylene oxide sterilization are: 1) it is more expensive than heat; 2) more monitoring is required during each cycle; 3) it will not kill spores in crystals, 4) it is necessary to determine if residual ethylene oxide or its reaction products remain in the material; and 5) dirtiness decreases sterilizing efficiency (16,18). Ethylene oxide sterilization is most effective when the relative humidity is controlled at 40 percent and the temperature is maintained at about 50-60° C.

Sterility assurance

Methods for the evaluation of sterility are outlined in the USP (8). Two basic methods are described requiring either direct inoculation of the solid material into culture media or solubilization and filtration followed by incubation of the filter in the culture media. The latter method is reported to be more sensitive by 100- to 1,000-fold (9). Biological indicators consisting of test strips of bacterial spores can be substituted for the device. These test strips are available commercially. After sterilization the device or test strip is placed in soybean casein digest medium (incubated at 20-25° C) and in fluid thioglycollate medium (at 30-35° C) and incubated for 7-14 days (5-8).

The use of biological indicators is controversial since there appears to be a wide range of variation of commercial test strips sterilized in a steam autoclave at 121° C. For a more detailed discussion of this subject the reader is referred to reference (9).

However, it is difficult to assure sterility of a batch of product by endproduct testing only. Therefore, approaches which take sterility system design, product design and manufacture, and process validation into account are used to most effectively and optimally assure the sterility of a product to a given probability level. A systems approach of this type is presented in the "Medical Device Sterilization Monographs" (13).

After determining which sterilization method is to be used for a given product, the first step in assuring sterility is validation of the process. This focuses on the mechanical equipment specifications and performance of the sterilizing equipment and serves to establish the operational range, parameters and limitations of the process regardless of the product to be sterilized.

The second aspect of this systems approach is cycle development. At this stage, product characteristics, such as chemical properties, configuration, and the packaging system and materials are weighed in the selection of the sterilization cycle. The average bioburden on the items in a batch should be known from past manufacturing history for other lots of the product. The cycle is then developed to establish assurance of sterility to a probability of microbial contamination of 10^{-6} or less. A useful example of this procedure for steam sterilization is available (19). Where gas or liquid sterilization is used, testing for the presence of residual chemicals is also be necessary.

The final important aspect of sterility assurance is microbial control in the manufacturing environment. A microbial control program should monitor production environmental conditions and establish good housekeeping through scheduled microbial monitoring. This manufacturing control should be designed to assure that the bioburden (microbial load) of a product remains within limits that maintain acceptable probability of sterilization and to establish procedures for dealing with incidents where bioburden limits are exceeded.

Much of sterilization assurance falls within the realm of good manufacturing practices. However, laboratories which fabricate sterile devices for experimental work should also be able to document and assure the state of sterility of those devices.

ENDOTOXIN TESTING

Taking great care to ensure the sterility of a device does not ensure that it is pyrogen-free. A pyrogen is any substance that will produce a fever when injected into humans or animals. The most common class of pyrogens is derived from gram negative bacteria and referred to as endotoxins--since they are released when the bacteria are lysed.

Endotoxins are high molecular weight (106 daltons) lipopolysaccharides which are intimately associated with the bacterial cell wall (20). There is good evidence that endotoxin activates complement (20,21), the intrinsic coagulation pathway through activation of factor XII (Hageman factor) (22), aggregate platelets (23), and indirectly promotes generation of thromboplastic activity in leukocytes (24,25) through activation of complement. Therefore, it is necessary to ensure that all materials designed for parenteral use be endotoxin free. The most probable primary source for endotoxin contamination of materials is the water used to clean the device. The only way to effectively remove or eliminate endotoxins is to use dry heat at 180° C for 4 hours or 250° C for 30 minutes or to acid treat the material (8,12).

Some polymer materials may selectively adsorb endotoxin. These include polypropylene, polyethylene, and fluorocarbon polymers (26). Since extreme dry heat and long-term acid exposure are difficult if not destructive procedures, it is much easier to screen materials for endotoxin than attempt to remove it.

Currently, the only official test for endotoxin is outlined in the USP (8). This test consists of injecting an eluate or extract from a device into the venous circulation of rabbits and measuring the febrile reaction of the rabbits. A material is considered pyrogen-free only if the rectal temperature of three rabbits injected with the extract does not show an increase of 0.06° C above the respective control temperatures. The test is extremely time-consuming and expensive, requiring preconditioned rabbits housed under strict temperature control and handled by trained personnel.

More recently, another acceptable test for pyrogenicity has been developed. That test is the Limulus amebocyte lysate (LAL) test (12,27) and is based on a distinctive property of the blood clotting mechanism of the horseshoe crab, Limulus polyphemus. The test is simple, fast and relatively inexpensive. However, there are some substances that have an inhibitory effect on this test, such as heparin (28). The Limulus lysate reagent is available commercially. Recently the FDA approved the use of the Limulus test if documented evidence is provided to demonstrate that this test system is as sensitive and reliable as the rabbit injection test. However, approval has been given only for specific applications (29).

References

1. T.Z. Loomis: *Essentials of Toxicology*, 2nd Ed., Lea & Febiger, Philadelphia, PA, 1974.
2. L.J. Casarett, J. Doull, Editors: *Toxicology - The Basic Science of Poisons* MacMillan Publishing Co., Inc., New York, 1975.
3. G. Zbinden: *Progress in Toxicology*, Vol. 1, Springer-Verlag, New York, 1973.
4. J. Autian: "The New Field of Plastics Toxicology - Methods and Results," in *Critical Reviews in Toxicology* , *Vol. 2* (June), CRC Press, Cleveland, 1, 1973.
6. R. Lefaux: *Practical Toxicology of Plastics*, CRC Press, Cleveland, 1968.
7. FDA Contract No. 223-73-5231, Quarterly Report #15-16; Part II. Round Robin Evaluation of Primary Acute Toxicity Screening Protocols.
8. United States Pharmacopeia, XIX, 1975.
9. A. Beloian: "Methods of Testing for Sterility and Efficacy of Sterilizers, Sporicides and Sterilizing Processes", in: *Disinfection, Sterilization, and Preservation*, S.S. Block, ed, 2nd Ed. 11, 1977.
10. R.R. Ernst: Sterilization by Heat, in: *Disinfection, Sterilization, and Preservation*, edited by S.S. Block, 2nd Ed. Lea and Febiger, Philadelphia, 481, 1977.
11. D.F. Williams, R.Roaf: *Implants in Surgery*, W.B. Saunders Co., Ltd., Philadelphia, 186, 1973.
12. J. Nelson: A Comparison of *In Vivo* and *In Vitro* Methods for Detection of Endotoxin, Masters Thesis, University of Utah, 1973.
13. Health Industries Manufacturers Association Report No. 78-4.1-3, 1978.
14. B. Bloch, G.W. Hastings: *Plastics in Surgery*, Charles C. Thomas Publisher, Springfield, 145, 1967.
15. Modern Plastics Encyclopedia, McGraw-Hill, N.Y. 1977/78.
16. G.B. Phillips, W.S. Miller: "Sterilization", in: *Remington's Pharmaceutical Sciences*, J.E. Hoover, ed., 15th Ed., Mack Publishing Co., Easton, 1389, 1975.
17. S.D. Bruck: Sterilization Problems of Synthetic Biocompatible Materials, J. Biomed. Mater. Res. *5* , 139, 1971.
18. R.R. Ernst: Sterilization by Means of Ethylene Oxide, *Acta Pharmaceutica Suecica*, *12*, suppl., 44, 1975.
19. "Guidelines for Sterilization of Intraocular Lenses by Manufacturer". FDA, Bureau of Medical Devices, Silver Spring, MD.
20. O. Westphal: Bacterial Endotoxins, Int. Archs. Allergy Appl. Immun. *49*, 1, 1975.
21. O. Luderitz, C. Galanos, V. Lehmann, M. Norminen, E.T. Rietschesl, G. Rosenfelder, M. Simon, O. Westphal: Lipid A: Chemical Structure and Biological Activity, J. Infect. Dis. *128*, 517, 1973.
22. D.C. Morrison, C.G. Cochrane: Direct Evidence for Hageman Factor (XII) Activation by Bacterial Lipopolysaccharides (Endotoxin), J. Exper. Med. *140*, 797, 1974.

23. J. Hawiger, A. Hawiger, S. Timmons: Endotoxin-Sensitive Membrane Component of Human Platelets, Nature, 256, 125, 1975.

24. J. Niemetz, D.C. Morrison: Lipid A as the Biologically Active Moiety in Bacterial Endotoxin (LPS) - Initiated Generation of Procoagulant Activity in Peripheral Blood Leukocytes, Blood 49, 947, 1977.

25. F.R. Rickles, P.D. Rick: Structural Features of *Salmonella typhimurium* Lipopolysaccharide Required for Activation of Tissue Factor in Human Mononuclear cells, J. Clinical Invest. 59, 1188, 1977.

26. N.S. Harris: Process for Removing Endotoxin from Biological Fluids, U.S. Patent No. 3,959,128 1976.

27. R.J. McAuley, R.D. Ice, E.G. Curtis: The Limulus Test for *In Vitro* Pyrogen Detection, Am. J. Hosp. Pharm., 31, 688, 1974.

28. J.D. Sullivan, S.W. Watson: Inhibitory Effect of Heparin on the Limulus Test for Endotoxin, J. Clin. Micro. 2, 151, 1975.

29. Federal register, 42: 57749, November 4, 1977.

CHAPTER VI

REFERENCE MATERIALS

Rationale

Even with well-standardized and relatively detailed protocols, the inherent differences among laboratories and the unevenness in the training and technical skills of various individuals often leads to a situation in which materials characterization procedures as performed in different laboratories yield results that differ in accuracy as well as reproducibility. Since these measurements form the basis of the subsequent judgments as to the compatibility of the material with blood, it is extremely important to eliminate systematic errors to the greatest extent possible and to assess the reliability and reproducibility of measurements made by different groups. Moreover, as new test methods are proposed, their proper evaluation will require that materials used in their calibration and development be well enough defined that the capability of the new method can be reasonably assessed.

Reference materials, used in conjunction with reference test methods, represent a useful approach to dealing with these problems. For a measurement process to be meaningful and to provide a confident basis for decisions, it must be capable of producing numerical values of the properties under test that are compatible throughout the measurement community. Measurement compatibility implies that all laboratories within the discipline area are capable of, and in practice do, obtain values for the properties of a given material that are identical within some agreed upon uncertainty. The use of "true values" not only means that measurements can be compared at a given time, but also ensures that data obtained at different times can be compared. In that respect, the development of reference materials minimizes the need for repeated calibrations as test systems change.

The Working Group has therefore concluded that the NHLBI should develop a set of reference materials for use in conjunction with physicochemical characterization of biomaterials. It is important that these materials be available in large enough amounts to supply the needs of investigators over several years; that they be homogeneous in their properties, which will usually require that they be synthesized in a single lot; and that they be characterized as carefully and exhaustively as is possible within the present state of the art.

The reference materials should be available in tubing, spherical particle, and sheet form to provide flexibility to users. If the material must be used in forms other than the one which is supplied, it will be necessary to recertify its properties following its fabrication since such processing can introduce impurities and/or change the morphology of the polymer. Materials should be available from a central supplier, possibly an NHLBI contractor or subcontractor, at modest cost with a complete characterization data sheet.

Characterization data should include the level I and II testing data. For bulk data, this would include molecular weight distribution, glass transition temperature, degree of tacticity, polymerization conditions, residual monomer and catalyst levels, and degree of branching, if any. Other bulk data should include the refractive index at common wavelengths (including 6,328 Å) and UV, visible, and IR absorption spectra. Swelling and solubility information should also be supplied, including various solubility parameter determinations. Mechanical data should include hardness and typical stress-strain tensile relations, specifying such parameters as elastic modulus. Surface characterization should include advancing and receding contact angles, critical surface tension value, surface composition (preferably by X-ray photoelectron spectroscopy), and SEM documentation of surface smoothness. Toxicity evaluations should be conducted to establish the absence of leachables and evidence of inertness.

Materials synthesized and characterized as outlined above must be prepared in quantity and properly packaged for storage and shipping. Since all materials for use as controls must eventually function in the aqueous physiological environment, packaging in distilled, deionized water in glass bottles or ampules should be considered where feasible. This technique would prevent condensation of volatile impurities at the air/solid interface and would preclude formation of toxic compounds like ethylene chlorohydrin if storage in physiological saline were to be followed by ethylene oxide sterilization.

Recommendations

In choosing appropriate reference materials, the working group focused on developing a set of materials that could be synthesized to exhibit a well-defined set of properties that would be likely to span the spectrum of properties of candidate materials. The actual compatibility of the materials with blood was a secondary consideration as was the utility of the materials in blood-material interaction testing. This was consistent with the working group's goal of establishing calibration and reference standards of primary use in physicochemical characterization. The materials recommended are described in the following sections.

Polyethylene

Polyethylene is available either as a linear or branched polymer and is easily fabricated into all required shapes. Moreover, it can be cast by mold injection into materials having well-defined surface textures. Because it contains no plasticizers or other leachable components and is relatively homogeneous in its properties, it is particularly suitable as a reference material and, indeed, it has the added avantage that it already exists in reference material form and has been well-characterized. It is translucent, a necessary requirement for certain of the test protocols in which it would be used.

Polyethylene is also a typical simple hydrocarbon; therefore, information obtained with it will be applicable to certain materials such as polyolefin rubber and polypentadiene, which have attracted some interest as potential blood compatible materials.

Polyethylene can be treated by glow or corona discharge to change its surface characteristics in a systematic way, varying the percentages of ethylene, acetylene, hydroxyl, and carboxyl bonds. Thus, it can be provided with a broad range of surface characteristics to determine the sensitivity of surface characterization procedures and to determine the importance of these characteristics in blood-material interactions.

FEP Teflon

This is an easily sterilizable, very low interfacial energy material. It is less crystalline that PTFE but, like other fluorocarbons, it is relatively nonreactive in pure preparations and is exceptionally resistant to contamination. It is extrudable so that it can be fabricated in appropriate forms and transparent so that it can be used conveniently in a number of test procedures.

Low energy surfaces--particularly various kinds of fluorocarbons--have been used widely in cardiovascular applications; thus, FEP Teflon will provide a reference material close in properties to an important class of cardiovascular biomaterials. Moreover, for experimental purposes, its surface properties can be changed by glow discharge treatment, thus providing a controllable and variable range of properties.

FEP Teflon has further advantages as a reference material since it is rich in fluorine, a useful calibration peak for ESCA and other surface analysis techniques. Moreover, because it has no hydrocarbon bands, it provides a noninterfering substrate for studying the adsorption of blood constituents, an important aspect of blood-material interaction studies.

Filler-free Polydimethyl Siloxane

This is a relatively clear, readily available material considered relatively blood compatible and presently used in certain blood oxygenation devices. It exhibits surface properties characteristic of closely packed methyl groups.

It is a homogeneous, essentially noncrystalline material, although in contrast to the other reference materials being recommended, it is somewhat elastomeric. It is also distinct from the others in that it absorbs lipids and can therefore be used to simulate a phenomenon of concern in blood contacting materials.

Graphitic Carbon

This is a very useful reference material in that it exhibits a set of properties far different from the other three and approaching, in certain respects, the properties of metals and ceramics which may be used in cardiovascular applications. It has a high surface energy and is electrically conductive, although not so much so that it does not have certain characteristics of ceramics.

Its conductivity is an advantage in that it increases the flexibility of testing, preventing charge buildup which can interfere with certain surface characterization procedures, and allowing experiments such as voltage clamp experiments which would permit better controlled studies of the effects of surface charge and potential on blood-material interactions.

Graphitic carbon is easily sterilizable by either gas or steam and, while it is a rigid material, it can be machined into various test forms. It is also obviously related to other forms of carbon which are finding wide use in cardiovascular applications.

Natural Reference Materials

Treated biological materials such as the porcine heart valve and the bovine heterograft are being used in cardiovascular applications. These materials have not been considered directly in this report and many of the procedures recommended here will not be readily applicable to such materials. The development of tests appropriate for such materials is clearly a gap area. For that reason, the working group is not recommending at this time that a reference material simulating their properties be developed.

Testing of Reference Materials

As each of the above reference materials are developed, they should be subjected to a round-robin testing protocol in which all the level I and level II tests recommended in this report are performed. This will serve as an initial intercalibration of the results of a number of laboratories and will also serve to establish the practicability, time investment, and cost of the entire battery of tests being proposed herein.

CHAPTER VII

RECOMMENDATIONS FOR FUTURE EFFORTS

Rationale

In the course of developing this report, the working group identified a number of areas in which further work is necessary to improve our understanding of the behavior of materials intended for use in cardiovascular applications. Certain of these efforts are necessary to develop more sensitive and informative methods for characterizing materials as we move toward a better understanding of the properties of a blood compatible substance. These are the level III tests discussed in the body of this report. Others are necessary to ensure that those procedures are sensibly and reliably used by the many groups and laboratories involved in materials development and device synthesis, and that the information obtained from them is made available in a timely and understandable fashion to the community of investigators and developers. Finally, the working group recognized that much remains to be done to understand the actual performance of materials--a consideration that goes well beyond initial characterization and the predictions of blood-materials interaction testing.

The recommendations which follow constitute a summary of specific items that fit in the above categories and have been described in greater detail in the body of the report. All of them represent directions in which it would be useful for the NHLBI to focus its efforts. Some, however, have a greater immediacy than others because they are limiting current progress in developing blood-compatible materials. These are identified in the next section. The efforts recommended in that section should be initiated at the earliest possible opportunity.

The remaining recommendations, presented in the subsequent sections, are more likely to gain in importance in the future as our state of knowledge advances and new approaches are needed to carry the development of cardiovascular materials and devices further forward.

High Priority Future Efforts

Development of Reference Materials

Development of reference materials for calibrating the reliability and accuracy of measurements from any laboratory and providing a basis for intercomparing the results obtained in different laboratories is a vital and immediate need. Polyethylene, FEP Teflon, filler free polydimethyl siloxane, and graphitic carbon are recommended. As these materials are developed, round-robin testing programs should be undertaken to verify the reproducibility of the characterization data.

Surface Charge Measurements

There is a great need to develop reliable methods for characterizing the electrical charge distribution on the surfaces of various kinds of materials and measuring such related properties as pH, acidbase behavior, etc. This is a significant gap area which has prevented resolution of an ongoing debate regarding the importance of electrical charge on blood-material interactions.

Protein Adsorption Studies

Investigations into the use of protein adsorption measurements for characterizing synthetic surfaces are needed. Efforts must be directed at selecting and characterizing appropriate proteins for such studies, at developing appropriate protocols for turnover and competitive adsorption experiments, and toward interpreting the meaning of the results of the experiments with respect to blood-material interactions.

Device Autopsy

One of the most useful approaches to determining the behavior of devices in clinical use and relating that behavior to material and device characteristics is to develop a procedure for recovering devices that have failed in use. This procedure should specify a rational and comprehensive scheme for examining these devices so that correlations can be developed between initial material properties and the changes in those properties brought about by the conditions of use.

Coordination and Communication of Research Efforts

It is necessary to establish mechanisms for coordinating and communicating the efforts of research groups in the areas of physicochemical characterization of materials and of blood-material interactions. In addition to the annual contractors' meetings, special workshops focused on particular issues which span the concerns of these two groups would be appropriate. These might be of the following kind: the role of material compliance or of surface charge in blood compatibility; the effect of surface properties on activation of the intrinsic clotting pathway, or on protein denaturation, etc.

Longer Range Future Efforts

Surface Heterogeneity

The question of more and more careful surface characterization may or may not take on great importance as our knowledge of blood-material interactions increases. At the moment, however, our ability to characterize the nature of surface heterogeneities at the submicron level is severely limited. We cannot answer questions such as the effect and importance of local "hot spots"--regions where the properties differ from the average value on the surface. Should it be necessary to look more closely at those questions, the program will have to step up efforts to develop techniques for quantifying such heterogeneities.

Fatigue and Environmental Stress Testing

Our ability to predict the effects of fatigue and environmental stress on surface and bulk properties is now quite limited and is a subject of ongoing investigations. As the NHLBI moves toward the development of devices designed for longer and longer use, our limited knowledge in this area and in the area of accelerated testing will become more constraining. Therefore, efforts in the direction of better understanding of and improved ability to measure environmental stress effects should be encouraged.

Molecular Surface Structure and Mobility

The mobility of side chains in the surface of a material and many features of the surface molecular architecture are essentially impossible to measure at the present time. Since these features may have an important role in blood-material interactions, it would be appropriate for the Program to support efforts aimed at increasing our ability to measure such properties.

Characterization of Natural Materials

The characterization of properties of materials that derive from natural sources (cross-linked heterografts, pseudointima, cultured cell linings) have not been addressed in this report and constitute a gap area. Some of the procedures discussed here may be applicable to those materials, but none have been validated in that respect. Efforts in this direction would be an appropriate undertaking of the program as would the future development of a reference material for such surfaces.

Reference Materials for Biological Testing

Reference materials and reference solutions that exhibit a range of responses to various biological tests do not now exist. Polyethylene is used as a negative control, but the absence of materials with a range of responses limits the intercomparability of the results of various laboratories and restricts the quantitation of results possible. Studies are necessary to determine and test appropriate candidate substances.

Testing for Cell Alterations

While toxicological testing is relatively far advanced and well-defined, chronic mutagenicity, teratogenicity, and carcinogenicity testing represent gap areas. Reliable, reproducible, and standardized protocols are not available and interpretation of results is still quite difficult. Since assurance of safety with respect to these considerations is clearly important, it would be useful for the NHLBI to support efforts in this area.

GLOSSARY

absorption	The reversible or irreversible transfer of a substance into the bulk of a solid or liquid
additive	Anything added to a polymerizing mixture which is not required to produce the polymerization itself
adsorption	The reversible or irreversible association of a substance with an interface
amorphous	Noncrystalline; a state in which the molecules are oriented randomly
antioxidant	An additive which improves the resistance of the polymer to oxidation
antistatic agent	Additives which impart some electrical conductivity to polymers, thus preventing the accumulation of electrostatic charges on finished articles
asepsis	A condition of sterility; freed from all forms of life
ASTM	American Society for Testing and Materials
auxotroph	A mutant lacking a specific factor for growth
bioburden	The number of microbes per unit volume of material
block copolymer	Copolymers in which long segments of a chain contain a single kind of monomer
branching point	The point of cross-linking of chains in a three-dimensional polymer
bulk	The portion of a material not containing an interface with another phase
carcinogenicity	The potential of a substance to induce a cellular mutation leading to the production of malignancies
ceramic	A material depending on ionic bonds for its cohesion--often an oxide, such as glass

composite	A material synthesized from two or more kinds of material to provide specific combinations of properties
compression modulus	A measure of the resistance of a material to changes in specific volume
contact angle	The angle between the solid surface and the liquid-air surface at the line of contact between liquid, air, and solid created when a liquid droplet is placed on a surface
copolymer	Polymer formed by the simultaneous polymerization of two or more monomers
creep	The irreversible dimensional change, with time, of a material under force loading
crystalline	A solid substance exhibiting local molecular order or orientation
dilation	Increase in the specific volume of a material
domain	A region in a block copolymer having physical characteristics determined by one of the polymeric constituents
elastomer	An elastic or rubber-like material, i.e., a material that can be stretched reversibly
electrokinetic phenomena	Those phenomena that represent a coupling of flow and electrical phenomena, e.g. electrophoresis, streaming potential, electro-osmosis
endotoxin	A high molecular weight lipopolysaccharide pyrogen released during lysis by gram negative bacteria
ESCA	Electron spectroscopy for chemical applications or XPS
extractable	A solute that can be extracted
extraction	The process of removing a solute from one phase to another by placing the two phases in contact with each other
extrusion	The process of forcing a polymer melt through a die shaped to give a desired final form

fatigue	The phenomenon of mechanical stress failure in a material associated with cyclical stressing
gel permeation	A method of separation and analysis of macromolecules based on ability of the species to diffuse into and out of the micropores of the gel beads with which the chromatographic column is packed
glass transition	That temperature below which local polymer chain molecular motions cease and polymers exhibit glass-like properties
glassy polymer	A polymer exhibiting glass-like properties of hardness, stiffness, and brittleness
hardness	The ability of a material to resist indentation
heat capacity	The amount of energy required to raise the temperature of 1 gm of material 1° C
hemolysis	The rupturing of red blood cells
heterogeneous	A situation in which detectable local variations occur in some or all of the physicochemical properties of a material
homogeneous	A situation in which physicochemical properties are the same within measurement sensitivity at every point in the material
hydrophilic	Exhibiting a high affinity for or relatively high solubility in an aqueous phase; usually a polar species
hydrophobic	Exhibiting a low affinity for, or low solubility in, an aqueous phase; usually a nonpolar species
infrared (IR) spectrum	The characteristic transmission pattern produced when a sample is exposed to infrared radiation. It arises from the absorption of infrared energy of particular wavelengths by specific kinds of chemical bonding groups.
injection molding	A procedure in which a heat-softened material is forced from a cylinder into a relatively cool cavity to produce solid product of desired shape

interface	The region between two contiguous homogeneous phases. Molecules in this region are subjected to forces different from those in the bulk. The region is usually several molecular diameters in thickness.
isotropic	A term referring to a situation in which properties do not vary with respect to direction
leaching	Extraction of a solute from a solid by contact with a liquid
loss modulus	A measure of the energy lost to heat through viscous dissipation during oscillatory loading of a material
macromolecule	A molecule of high molecular weight
mass spectrometry	A method of analysis in which the molecules are ionized and their masses determined by their trajectories in electrostatic or magnetic fields, or by focusing techniques
mobility	The ability of a species to move through the polymer matrix
mold-release	A chemical compound applied to a mold surface to prevent the polymer from sticking
monomer	The small molecule that forms the repeating unit in a polymer
mutagenicity	The potential of a material to cause mutations in the germ cells of an animal, affecting its offspring
nuclear magnetic resonance (NMR) spectrum	The spectrum produced by the absorption of radio frequency energy by atomic nuclei subjected to magnetic fields
optical birefringence	Change in the index of refraction of a material with direction, causing a rotation in the plane of incident polarized light
osmotic fragility	The tendency of cells to lyse when suspended in a medium whose total solute concentration is lower than that of the intracellular solution
Pascal	A unit of pressure equivalent to 1 Newton meter^2 or 0.000145 psi

penetration depth	The depth beneath a surface "sampled" in various kinds of analytical techniques
permeation	The process by which solutes pass through a polymer phase. It is a function both of solubility and diffusivity of the solute.
plasticizer	An additive that increases the flexibility of a polymer, lowers its melt viscosity, and makes it easier to process at lower temperatures
PMMA	Polymethyl methacrylate
polar group	A group containing a dipole moment or ionic charge
polymer	A macromolecule built up by the repeated bonding of small chemical units called monomers
polymerization	The chemical chain reaction that results in the formation of a polymer
PP	Polypropylene
psig	Pressure unit; pounds (force) per square inch gauge, i.e., pounds per square inch above ambient pressure
PTFE	Polytetrafluoroethylene
pyrogen	A substance that will produce a fever when injected into humans or animals
pyrolysis	The breaking of chemical bonds by heat
Raman spectrum	The scattering spectrum produced by inelastic collisions between an incident beam of monochromatic radiation and the molecules of a sample. The spectrum is characteristic of the dipole moment induced in the molecule.
relaxation	The time-dependent response of a material to an instantaneous imposition of a constant stress or strain
shear modulus	A measure of the resistance of a material to shear deformation
shear rate	The rate at which deformation is induced by a shear force. Units are sec^{-1}.

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shear stress	A stress tengential to the area of application
solvation	The process by which molecules become surrounded by solvent molecules
stabilizer	An additive that inhibits physical or chemical degradation of a material
sterility	Complete destruction or removal of all forms of life from a material
storage modulus	A measure of the energy stored reversibly (elastically) during oscillatory loading of a material
strain	The extensional deformation of a material from a resting, or unloaded state. It is usually measured as a fractional extension and is thus dimensionless.
strain rate	The rate at which extensional strain is induced in a material. Units are sec^{-1}.
streaming potential	The axial electrical potential difference generated by the flow of an ionic liquid past a solid surface of finite surface charge
stress	The amount of force per unit of area acting on the actual surface of a material or on any imaginary surface within the material
surface	In common usage, an interface in which one of the contiguous phases is a gas
surface energy	The additional energy per unit area of molecules at a material surface
surface tension	The tensile force developed at a liquid surface as a result of the asymmetric molecular interactions to which molecules in the surface are subjected. Units are dynes/cm.
surfactant	A substance that lowers the surface tension of a solution; some mobile components in polymers may display this property after migration from the bulk to the polymer surface
tensile stress	A stress perpendicular to the area of application and tending to extend the material in a direction parallel to the stress

teratogenicity	The potential of a material to effect alterations in fetus during development
three-dimensional polymer	A polymer in which the chains are branched, forming three-dimensional networks
torsional modulus	A measure of the resistance of a material to torsion or twist
toxicity	The tendency to produce a deleterious effect on animals or men, including alterations in physiological responses, tissue or organ damage, or death
transfer agent	An additive that interacts with a growing polymer chain during polymerization to initiate a new polymer chain
two-dimensional polymer	A polymer made up of linear chains of monomeric units
USP	United States Pharmacopaeia
UV	Ultraviolet
velour	A surface composed of vertically oriented, tightly packed fibers attached to a solid substrate
viscoelasticity	Deformation behavior of a polymeric solid or liquid in which both reversible (elastic) and irreversible (viscous) responses to shear force loading and unloading occur
wettability	The characteristic of a surface that describes the ability of a drop of a particular liquid to spread on it
wide-angle X-ray diffraction	The X-ray scattering pattern produced at relatively large scattering angles and associated with local molecular order or crystallinity
XPS	X-ray photoelectron spectroscopy or ESCA
X-ray fluorescence	A method of elemental analysis which uses the characteristic X-ray spectra excited when a sample is irradiated with short-wave X-radiation

zeta potential	**The electrical potential measured at the "slipping plane" adjacent to a surface past which a fluid is flowing. The slipping plane is the dividing plane between those ions adsorbed on the surface and those flowing.**

Appendix A

Particulate Contamination and Characterization

by D. L. Coleman

Particulate contamination on medical devices has not been officially recognized as a problem. The USP (1) mentions particulates only in regard to injectable solutions and, even there, only requires visual inspection. Even under the best of conditions the unaided eye can only detect particles in the 60-70 μm diameter range (2). Currently, the most demanding industry in terms of particle contamination is the electronics industry (3,4).

Surface adherent particles may come from virtually any source which is directly exposed to the material. This includes particles suspended in fluids used to clean the device, body dandruff from personnel handling the device (including hospital staff or lab personnel) and from the air. McCrone and Delly (5) estimate that an exposed, clean microscope slide will collect easily visible dust particles at a rate greater than 5,000 per hour in the average laboratory.

Detection methods for measuring and counting particles on surfaces routinely involve the use of the light microscope equipped with an incident light source. ASTM standards (6) clearly outline methods to determine particulate contamination 5 μm or greater in size. Less sophisticated tests include the water-break test and the atomizer test to detect oil films on surfaces; freshly cleaved mica is used as a control (7). Identification of smaller particles requires more elaborate and expensive techniques such as scanning electron microscopy, transmission electron microscopy, X-ray microanalysis, and others [see Volumes 1 through 4, (5)].

Adhesive forces between substrate and particle can reach unbelievable magnitudes especially as the particles become smaller. A 50 μm particle will adhere to a glass slide with forces ranging up to 1.8 dynes (7). The dominant forces involved in the adhesion process of particles in the size range below 50 μm are mainly intermolecular forces. Static electrical forces can become important in some cases, e.g., certain polymers. The magnitude of these forces is brought into focus by the fact that it would require 20,000 g in air to remove a 5 μm particle from a surface (3). It seems reasonable that particles below 5 μm will not be removed except by extraordinary means.

Prevention of particulate contamination is, of course, the best approach to minimizing this problem. Working in a laminar flow filtered air environment or a special clean room will help eliminate much of the problem. Personnel should wear special clothing and be trained in clean room techniques for such a system to be effective (8). Freon cleaning solvents have been found to be very effective in removing particulates and degreasing plastic and metal parts (3). Freon TMS is a Freon-methanol azeotrope which is reported to be very effective at removing particulates from surfaces (9). Also, T-WD602 solvent contains a surfactant and when used according to instructions (in combination with an ultrasonic cleaner) can be equally effective (10).

The removal of particulates smaller than 5 µm is extremely difficult. It is likely in the case of catheters and other devices that contact blood, that such particles could act as sites of thrombus formation or become emboli generators.

Basic scientific studies involved in the evaluation of materials for medical devices should at least exhibit awareness of the problems of sterilization, endotoxin contamination, and particulate contamination.

References

1. United States Pharmacopeia, XIX, 1975.
2. W.C. McCrone, J.G. Delly: The Particle Atlas, second edition, Vol. 1, Ann Arbor Science Publishers, Inc., 1973, p. 803.
3. D.A. Brandreth, R.E. Johnson: Particulate Removal in Microelectronics Manufacturing, preprint (1978).
4. A.H. Szkudlapski: Surface Cleaning Practice, in: Environmental Control in Electronic Manufacturing, edited by P.W. Morrison, Von Nostrand Reinhold, 1973, pp 181-206
5. W.C. McCrone, J.G. Delly: The Particle Atlas, 2nd edition, Vol. 1, Ann Arbor Science Publishers, Inc., 1973, pp 272-288.
6. ASTM, Measuring and Counting Particulate Contamination on Surfaces, (F24) 8 (1965).
7. J.L. Dwyer: Contamination Analysis and Control, Reinhold Publishing Corp., 1966.
8. M. Zief: Talking Through Glass Fibers - The Chemists Role, Chemtech., October (1978) 610-614.
9. E.I. Dupont De Nemours and Co., Freon Products Division. Technical Bulletin, No. FST 5-J.
10. E.I. Dupont De Numours and Co., Freon Products Division, Technical Bulletin, No. FST-5E.

Appendix B

Critical Surface Tension

by R. E. Baier and C. K. Akers

An important requirement in biomaterials research is for an extremely sensitive and reliable method of monitoring the surface properties of coatings and substrates. Perhaps the fastest and most sensitive technique known for identifying changes in surface constitution of any material is based upon contact angle measurements. Using air-dried specimens, critical surface tensions are measured from the slowly advancing contact angles of drops of pure liquids of known surface tension.

Contact angle methods have been developed extensively over the past four decades by Zisman and coworkers at the U.S. Naval Research Laboratory. A large body of reliable data has been accumulated and a vast literature exists correlating contact angle data with surface properties of liquids and solids. The Critical Surface Tension, γ_c, which is a useful parameter which can be obtained by measurement of the contact angle (figure 14) of each of a series of homologous liquids on the test material and a graphical treatment of the contact angle data (figure 15). Although this value is precisely that for most liquids the values fall along the same straight line or cluster closely around it in a narrow rectilinear band. Other interpretations and treatments of contact angle data are being developed and used.

A most important consideration for screening materials for potential contact with blood or tissue is that the Critical Surface Tension values are empirically related to the surface constitution. Even small changes in the outermost atomic layer are reflected in a change of Critical Surface Tension, while other properties might remain essentially unchanged. For example, a simple hydrocarbon surface (like that of polyethylene) exhibits contact angles leading to a Critical Surface Tension of about 31 dynes/cm. Gradual replacement of hydrogen atoms in the surface by fluorine atoms gradually decreases the critical values to 19 dynes/cm (as observed with polytetrafluoroethylene). Conversely, gradual replacement of surface hydrogen with chlorine atoms leads to an increased Critical Surface Tension gradually approaching 41 dynes/cm (as with polyvinylchlorides).

Figure 14 defines the contact angle of a liquid on a solid surface. The angle is measured through the liquid phase so that a contact angle of zero degree ($\theta=0^\circ$) indicates that spontaneous spreading of the liquid takes place; larger equilibrium contact angles ($0^\circ<\theta<180^\circ$) indicate poorer, or incomplete, surface wetting by the liquid phase.

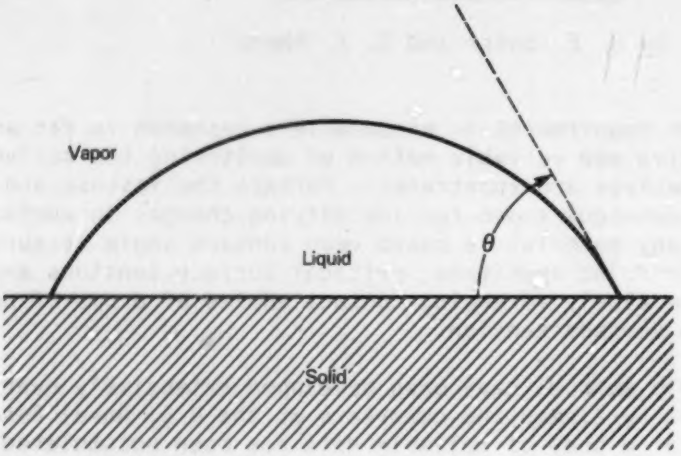


Figure 14. Definition of the contact angle θ.

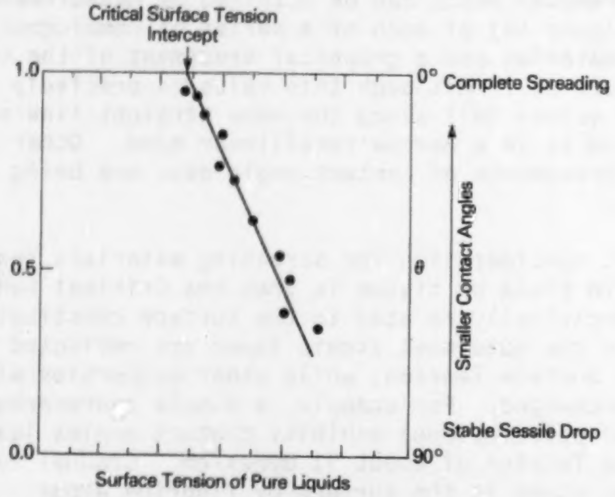


Figure 15. A representative Zisman Plot.

The Critical Surface Tension, γ_C, of a polymer can be deduced from a Zisman plot, illustrated in Figure 15. In a Zisman plot the cosines of the contact angles for a variety of pure liquids on a given solid are plotted against the liquid/vapor surface tensions of the liquids. It has been learned empirically that linear fit to the data points is most often obtained. Since the intercept at the cos θ = 1 axis is different for different surfaces, the intercept surface tension value is recorded as the Critical Surface Tension for the solid in question.

Appendix C

The Teflon Test

by R. E. Baier

Contact angle methods can also provide rapid and inexpensive answers to questions of surface cleanliness and stability. A simple, rapid method (the Teflon test) can be used to detect the surface activity of minute amounts of organic contaminants, often encountered as coating compounds.

The following diagram (figure 16) is a standard plot of the contact angles obtained for a series of pure wetting liquids on Teflon. To perform the test for surface contaminants, a drop of pure liquid--for example, water--is placed on smooth Teflon FEP film; its contact angle is recorded--for water this would be about 110°; the material having unknown surface properties is touched to the drop; if there are no transferable surface active constituents, the contact angle of the drop remains unchanged--a good test of the surface cleanliness is thereby completed; if surface active agents are present, the drop profile changes instantaneously to exhibit a new equilibrium contact angle reflecting its new surface tension; the surface tension which corresponds to the new contact angle can be deduced from the standard Teflon plot; the difference between the surface tension of the pure liquid and the surface tension of the contaminated liquid represents the surface activity (i.e., the spreading pressure) of the agent.

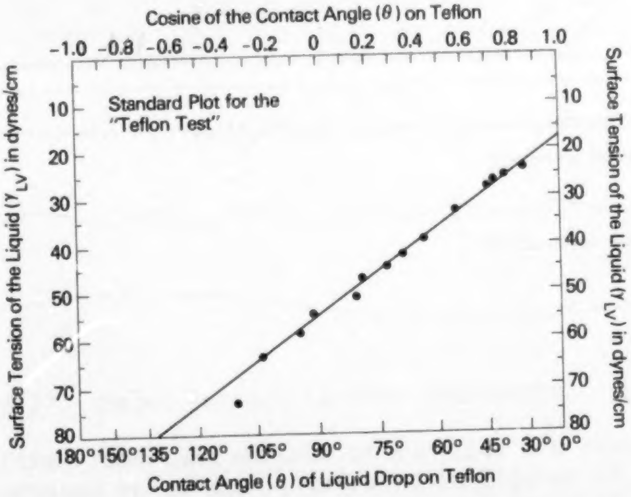


Figure 16. A standard plot for the Teflon test. The contact angles of Teflon with each of a series of pure liquids is presented. A change of the contact angle of Teflon with one of these liquids after the liquid has been exposed to a test material is indicative of the presence of leachable components in the test material.

Appendix D

Surface Texture

by E. Gasiecki

The surface profile of a synthetic or engineering material can be described with three major components (see figure 17):

- -the roughness of primary texture,
- -the waviness or secondary texture, and
- -longer term (lower frequency) errors of form.

Generally speaking, most surface metrification and observation methods readily identify the roughness and perhaps the waviness of a surface under study. The lowest frequency component, error of form, may have a wavelength so long that observing it makes roughness and perhaps waviness disappear by loss of resolution. The converse is, of course, equally true. Observations made using light microscopy and the scanning electron microscope suffer from this particular shortcoming. Thus, SEM and light microscope observations, while adequate (for some materials) in characterizing roughness and perhaps waviness, probably do not adequately address errors of form.

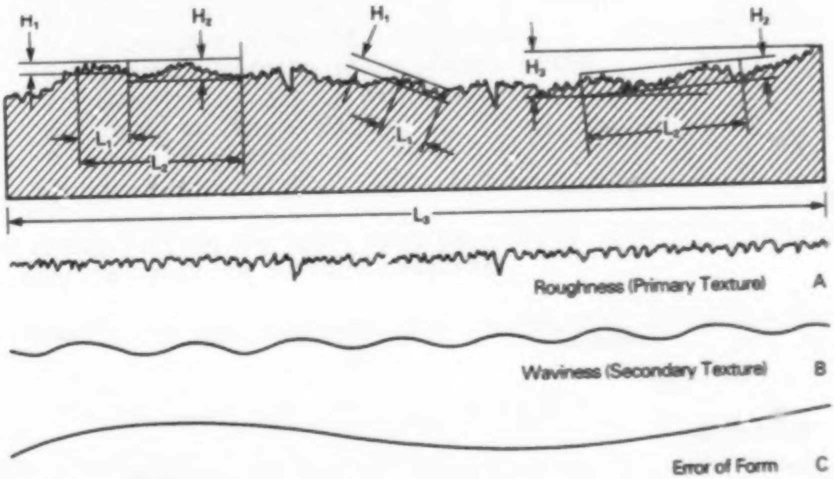


Figure 17. Major surface texture components.

Other important moments of surfaces are skewness and kurtosis, as discussed below and illustrated in figures 18 and 19. Since the relationship of roughness, waviness and error of form is complex, filtering operations must be used to separate these components one from another.

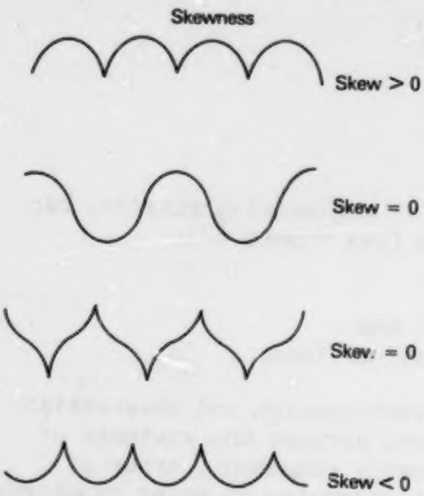

Skewness
Skew > 0
Skew = 0
Skew. = 0
Skew < 0

Figure 18. Skewness

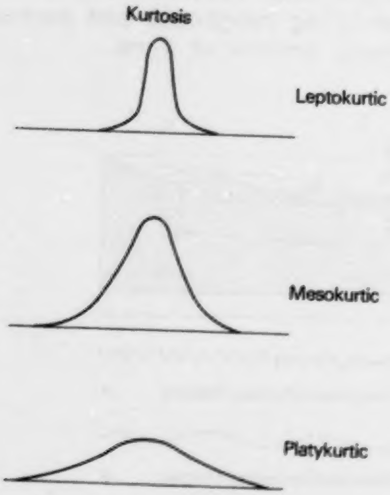

Kurtosis
Leptokurtic
Mesokurtic
Platykurtic

Figure 19. Kurtosis

It is obvious that sampling or cutoff lengths required to quantify roughness, waviness and error of form are vastly different, varying in direct proportion with some multiple of the component's frequency. While some of these components may be meaningful in characterizing a surface for some applications, it is not expected that error of form or perhaps waviness (if long-term) will be directly meaningful for application in characterizing the effect of surface roughness on blood interactions. The effect of surface roughness on thrombus formation is expected to be independent of error of form and, perhaps, waviness. Thus, it is probably most useful to concentrate on moments and derivatives used to characterize the roughness of a specimen surface. Elements of roughness considered at this point to be potentially important to thrombus formation include frequency, lay, skewness and kurtosis, and maximum and minimum height value.

The frequency of the roughness is the number of peaks per unit length of the surface. Lay is the directionality of a surface with repeating textural units. Skewness is a measure of the lack of symmetry about the mean of the distribution of heights as illustrated in figure 18. Kurtosis, illustrated in figure 19, is an indication of the peakedness of a distribution. Relative to surface measurement, leptokurtic distributions are characteristic of surfaces in which heights tend to cluster near a mean level with only occasional extreme excursions. In platykurtic distributions, there is more of a tendency toward frequent extreme swings from high to low elevations, with relatively little "dwell" near the center line.

Various measures and derivatives thereof are used to characterize surface geometries. There are currently 10 common parameters used in characterizing a material's roughness:

1. arithmetic average roughness
2. root-mean-square roughness
3. maximum peak-valley roughness
4. ten-point height
5. average peak-valley roughness
6. average spacing of roughness peaks
7. Swedish height of irregularities
8. bearing length ratio
9. leveling depth
10. waviness height.

The above listing includes most parameters used in industrialized nations to describe surface textures.

Arithmetic average roughness (AA) is computed using the formula

$$\text{AA or } R_a = \left(\sum_{i=1}^{n} |y_i - \bar{y}| \right)/N$$

where y_i is the i^{th} height value, N is the total number of data points, and

$$\bar{y} = \left(\sum_{i=1}^{N} y_i \right)/N, \text{ the mean.}$$

Root-mean-square roughness (RMS) may be defined thus:

$$\text{RMS} = [(\sum_{i=1}^{N} (y_i - \bar{y})^2/N]^{1/2} .$$

The frequency of roughness elements or peaks may be defined by computing the average spacing (A_r) of roughness elements or peaks. If A_{rn+1} is the spacing between peak n and peak n+1, then

$$A_r = (\sum_{i=1}^{n} A_{ri})/N$$

yields the average distance between the peaks or the frequency.

The moments of a distribution are defined as expected values weighted according to the probability of encounter in a random process. The first moment of a distribution is the mean ($\bar{y}$, see above). The second moment is the variance and measures the dispersion of a distribution. Skewness and kurtosis are two other important functions or moments. Skewness may be expressed as the nondimensional ratio.

$$\text{Skewness} = \frac{M_3}{M_2{}^{3/2}}$$

where

$$M_3 \text{ (the third moment)} = \frac{1}{N} \sum_{i=1}^{N} (y_i - \bar{y})^3$$

and

$$M_2 \text{ (the second moment)} = \frac{1}{N} \sum_{i=1}^{N} (y_i - \bar{y})^2 .$$

Kurtosis is expressed by

$$\text{Kurtosis} = \frac{M_4}{M_2^2}$$

where

$$M_4 \text{ (the fourth moment)} = \frac{1}{N} \mathop{}_{i=1}^{N} (y_i - \bar{y})^4$$

and M_2 is the variance or second moment.

Thus, from simple measurements amenable to digitization, a number of parameters may be defined that are potentially useful in describing the texture-based contribution to blood-materials interactions.

Reference Standards

When there are needs to investigate a material's surface textural contribution to thrombus formation under varying conditions of flow, experiments designed to accomplish this task should involve at first a screening set to determine whether differences can be detected when blood or blood fractions encounter grossly differing surface roughnesses. More subtle effects, springing from differences in lay, skewness and kurtosis, should be reserved for later study in experiments using closely characterized surfaces. In addition to differences in skewness, inversion of skewness should be examined by using mirror image replicas.

To aid in such work, there are readily available replicas of surface standards in a variety of roughnesses. In the surface metrology terms introduced in the preceding pages, the available surface standards may be described as

honed--linear lay; skewness >0; leptokurtic;
ground--linear lay; skewness ~ 0; meso- to platykurtic;
Blanchard--lay is angular in both directions; skewness ~ 0; meso- to platykurtic;
shaped (turned)--highly directional lay; skewness = 0; leptokurtic;
profiled--lay is angular in both directions; skewness ~ 0; lepto- to mesokurtic;
side or end milled--circular lay; skewness ~ 0: lepto- to mesokurtic;
periphery milled--linear lay; skewness ~ 0; lepto- to mesokurtic;
cast finishes--no lay; skewness ~ 0; lepto- to mesokurtic;
grit blasted surfaces--no lay; skewness ~ 0; lepto- to mesokurtic;
shot blasted surfaces--no lay; skewness ~ 0; lepto- to mesokurtic; and
EDM surfaces--no lay; skewness >0; meso- to platykurtic.

Surface roughness standards are commercially available. The standards from one manufacturer are electroformed nickel replicas of original manufacturing surfaces. Typical replicas supplied are

> Machined surfaces including honed, lapped or polished, ground with periphery of wheel, ground with flat side of wheel (Blanchard), shaped or turned (these are equivalent), side milled, end milled, or profiled, and milled with periphery of cutter. These specimens include a high degree of lay and hence represent a repeating surface.
>
> Cast finishes in roughnesses equivalent to die castings ($\sim 20\mu$in. RMS) through normal ferrous green sand castings (560-920μin. RMS).

Grit blasted surfaces (32-1,000μin.).

Shot blasted surfaces (32-1,000μin.).

Electrical discharge machined surfaces (16-250μin.).

The above standards except the machined surfaces, do not have lay and therefore are completely random.

Appendix E

Protocol for Contact Angle Measurements at the Polymer-Water Interface

by R. N. King

Contact angles at the polymer-water interface are determined by immersing the sample in double distilled water and measuring the height and diameter of both air and octane bubbles in water (figure 20). In addition, dodecane is often used as a third liquid as a correlative check on the octane data.

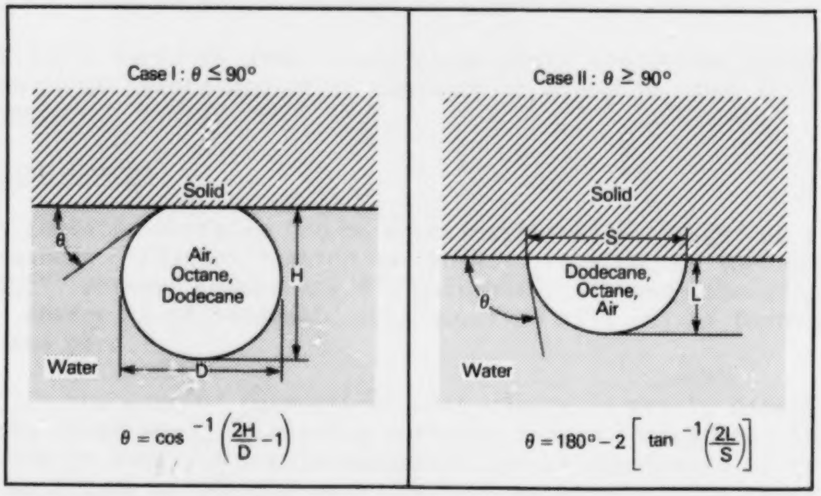


Figure 20. Measurement of contact angles at the polymer-water interface.

The bubbles of air or liquid are introduced to the immersed sample surface using a micro liter syringe equipped with a U-shaped needle. The highest purity octane and dodecane available (99 percent or better) should be used. The height and diameter are measured by use of micrometers which manipulate a stage holding the sample immersed in water. The bubbles are observed through a microscope using a 20x objective with a long working distance and a 15x eyepiece equipped with a fine crosshair reticle. The bubble is manipulated across the crosshairs and the dimensions are read directly from the micrometers. The sample box is back-illuminated by a variable light source. The bubble volume is approximately 0.1 to 0.2 μl. The bubble volume is minimized in order to avoid buoyancy effects. The bubbles are applied to the surface by forming a bubble at the tip of a microsyringe and then tapping the syringe body with the index finger to allow the bubble to dislodge and float from the needle tip up to the water-sample interface. Figure 20 schematically illustrates the geometry of the contact angle measurement. The contact angles are calculated using the equations.

$$\theta = 180° - 2[\tan^{-1}(\frac{2L}{S})]$$

for angles greater than 90° and

$$\theta = \cos^{-1}(\frac{2H}{D} - 1)$$

for angles less than 90°.

Appendix F

Measurements of Surface Electrical Properties

by V. DePalma

Electrical parameters of solid surfaces may eventually be correlated with the earliest phases of blood-material interactions. The electrical parameters that are described here are

1) Surface charge
2) Surface potential difference
3) Rest potential
4) Zeta potential (streaming potential).

Most solid surfaces under study allow these electrical parameters to be measured before and after exposure to blood or other biological phases.

Surface Charge

Isolated surfaces can be electrostatically charged by a number of methods. Electrification by contact, rubbing, or through permanently charged groups are all possible. Although the origin of this charge is of interest, this question will not be further addressed here.

In the case of conductors, this charge is easily dissipated. On the other hand, insulating surfaces remain charged for long periods of time. A simple apparatus can be fabricated to determine the magnitude of the charge on various materials. The required apparatus is essentially a Faraday cup which is electrically isolated from a metal container in which it is centrally located. A sketch of one such device is shown (figure 21).

The inner cup shown is connected electrically to an electrometer using a very short piece of coaxial cable. The shielded part of the cable connects to the outer shield of the assembly and to the ground post of the electrometer.

When a charged insulator (the specimen) is placed on the hook attached to the inner cover and the specimen is lowered into the Faraday cup (this cup being previously grounded using a grounding button on the assembly) it will charge the inner cup by induction. On the other hand, if the specimen is a conductor, it will charge the Faraday cup by contact. The charges transferred, or induced on the inner cup, result in a voltage, V, which is measured with the electrometer.

The expression relating the charge to the voltage is

Q = CV where

Q is the charge in coulombs on the specimen
C is the total capacitance in Farads
V is the measured voltage in volts.

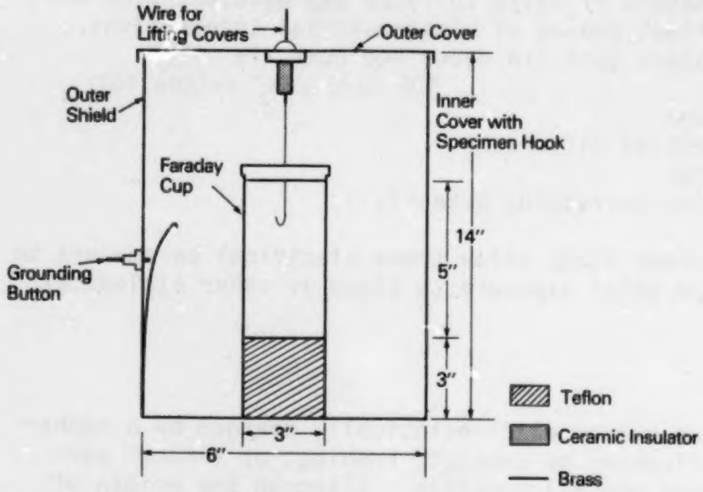


Figure 21. Faraday cup for measurement of surface charge.

Surface Potential Difference

The key to understanding the information which is obtained from a measurement of the surface potential difference is in the word, difference. This means that the voltage between two phases is measured and then, after introducing a charge to one of the phases (e.g., adsorption of a protein), this voltage is remeasured and the difference between this and the first value is recorded. Since the measured voltage is developed entirely in the space between two phases, none of the uncertainties of the phase boundary potentials is involved.

Consider the surface potential betwen two clean dissimilar metal surfaces as determined using an ionizing electrode technique described below. The voltage recorded in this case is due to the difference in work function between the two metal surfaces. If a film is placed on one of the metals, it will result in a different measured potential. The difference in potential between the clean and the film-coated metals has been related to the electrostatic dipole moments (both permanent and induced) in the film. It has been found that even nonpolar materials exhibit a surface potential difference due to induced polarization of the film by the electrostatic field generated by the metal on which the film is placed. In the case of the surface potential between a metal and a liquid, a similar potential is developed when a film is spread on the liquid. In this case, there may be a contribution to the surface potential difference due to a reorientation of the liquid phase dipoles as a result of an interaction between the film and liquid molecules. Although surface potential measurements have often been used in the investigation of the orientation of molecules spread on a liquid by relating the measured electrostatic dipole moment with computed values deduced from molecular structure, the unknown contribution of the restructured liquid phase to the surface potential difference makes the results difficult to interpret.

Considering the surface potential difference, ΔV, to result from the displacement of charge as in an electric dipole, μ, leads to the equation attributed to Helmholtz, which is

$$\Delta V = 4\pi\mu n$$

where

ΔV is the surface potential difference in volts absolute

n is the number of molecules per cm^2

μ is the electrostatic dipole in Debyes.

This equation can be modified to the more convenient

ΔV in millivolts
A as the area per molecule in $Å^2$/molecule
μ as the electrostatic dipole in milli-Debyes, to give

$$\Delta V = 12\pi\mu / A.$$

Actually, μ is only the component of the electrostatic dipole perpendicular to the surface. It can be resolved into two parts, $\mu_p + \mu_i$, in which μ_p is the permanent part of the dipole and μ_i is the induced part. Using this expression for μ in the above equation gives

$$\Delta V = 12\pi(\mu_p + \mu_i)/A.$$

In this expression, the two parts of the electrostatic dipole term correspond to their components perpendicular to the surface of the film. Hence, a dipole orientational term (i.e., the angle of the dipoles relative to the surface) must be known in order to use ΔV for a determination of overall molecular orientation.

Up to this point, the film of material either at an air/liquid interface or adsorbed on a metal has been considered electrically neutral. This is not always the case, since some films contain ionized groups which will also contribute to the measured surface potential difference. This has been included in the theory by adding to the above equation a term, Ψ, which represents the potential difference across the film because of the double layer for surfaces, films on solutions, and ionized groups within the films. With this term incorporated, the expression for ΔV is

$$\Delta V = 12\pi(\mu_p + \mu_i)/A + \Psi.$$

This equation, in principle, can be used to gain information on the orientation of the molecules within a film, provided the kind and strength of the electrostatic dipoles present and the packing density of the molecules are known, and some estimate of Ψ can be made.

A typical instrument used to determine the surface potential of a metallic plate utilizes a sealed, gold-coated alpha particle radiation source to reduce the impedance between the test material and the measuring electrode. The radiation electrode is used also as the measuring electrode. Therefore, the surface potential recorded is the potential of the metal with respect to the gold surface of the radiation electrode. When the ionizing electrode used is an Americum 241 sealed source with a mean alpha particle range of 2 cm in air, supplied as a thin foil soldered to the head of a #6-32 bolt, the sensitive area for surface potential measurements is approximately the full area of the bolt head, or 0.635 cm diameter.

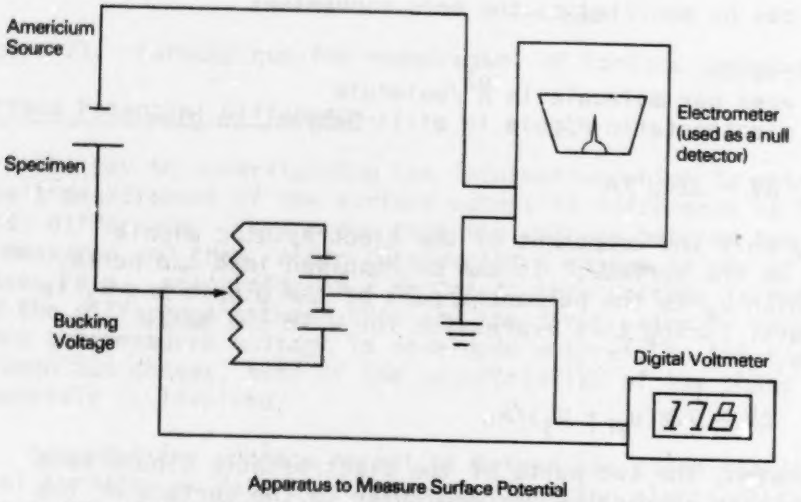

Americium Source
Specimen
Electrometer (used as a null detector)
Bucking Voltage
Digital Voltmeter
178
Apparatus to Measure Surface Potential

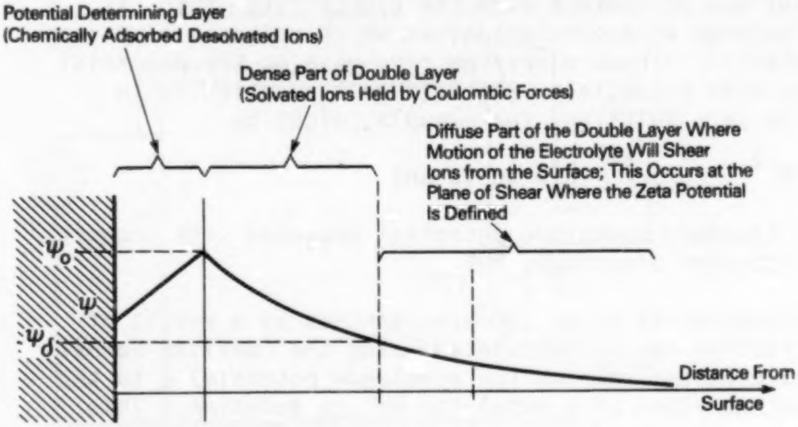
Figure 22. Apparatus to measure surface potential.

The measuring apparatus typically utilizes a null detector scheme to determine the surface potential. This is done by applying a voltage opposite in sign but equal in magnitude to the surface potential to be determined. Under this condition, a high impedance voltmeter connected between the radiation source and the specimen will be at null. An instrument to accomplish this measurement is illustrated schematically in figure 22. With this instrument the surface potential measurement is fairly insensitive to small changes in the separation between the radiation electrode and the specimen.

The reading on the digital voltmeter is equal and opposite in sign to the surface potential between the specimen and the radiation electrode.

Rest Potential

Any material in contact with an electrolyte has at its solid /liquid interface a potential determining layer (made up of desolvated ions chemically adsorbed to the surface) and a double layer which consists of a dense part (Helmholtz or Stern layer) and a diffuse part (Gouy layer). These separate regions of charge are indicated in figure 23 for a surface placed in an electrolyte.


Potential Determining Layer
(Chemically Adsorbed Desolvated Ions)
Dense Part of Double Layer
(Solvated Ions Held by Coulombic Forces)
Diffuse Part of the Double Layer Where Motion of the Electrolyte Will Shear Ions from the Surface; This Occurs at the Plane of Shear Where the Zeta Potential Is Defined
Ψ_o
Ψ
Ψ_δ
Distance From Surface

Sketch of Potential Near a Surface in an Electrolyte;
The Potential Is Shown in Arbitrary Units Along the Vertical Axis

Figure 23. Changes in potential near the surface of an electrolyte.

The potential-determining layer can make the potential, Ψ_o , positive or negative and depends on the specific potential-determining ions present. The case where the potential is increased is shown in figure 23. The electrical potential outside the layer of potential-determing ions always decreases. The ions in this layer are believed to be electrostatically adsorbed and only partially solvated. This reduces the potential Ψ_o to Ψ_δ. Outside of this layer is the diffuse part of the double layer consisting of positive and negative ions with a greater number having a sign opposite to the potential Ψ_δ. The plane of shear within the diffuse part of the double layer is where the zeta potential is defined.

Rest potential as discussed here is the potential of an electrochemical cell consisting of the material of interest in contact with an electrolyte, referenced to a Ag/AgCl electrode. The rest potential results from a corrosion reaction of the test material in the electrolyte. In many cases, the reaction is not reversible and there is a loss of material from the test material's surface. The discussion which follows gives a measure of the magnitude of the effect which would be observed under equilibrium conditions. Any electrochemical cell under study can usually be divided conceptually into two half cells. The first half cell may be considered to be the test material electrode in contact with the electrolyte. The second half cell is then the Ag/AgCl electrode in contact with the same electrolyte. Where the electrolyte contacts the test material's surface, an ionic double layer is set up from the material's surface and extends into the electrolyte. If the material is negatively charged, as proposed in many biomedical development efforts, cations from the solution are attracted to its surface. The concentration of these cations near the surface (called the Helmholtz layer) differs from that in the bulk solution. Outside of this layer is the diffuse layer which usually contains the probable plane of shear. The material surface in contact with the electrolyte, then, is considered to undergo an anodic oxidation which releases electrons to the bulk material. These electrons give rise to the potential measured as the rest potential. The half cell reaction for a semiconductor in salt solution, for example, might be

$$M = M^{+2} + 2\bar{e} \; ; \; E^{\circ} = 0.0 \text{ volts NHE}$$

where E° is the standard electrode potential measured with respect to the normal hydrogen electrode, NHE.

The concentration of metal ions in solution as a result of this anodic oxidation can be calculated using the familiar Nernst equation. This equation relates the electrode potential E to the equilibrium concentration of a metal ion M^{+z} in solution. This equation is

$$E_{M^{+z}} = E^{\circ}_{M^{+z}} + \frac{.06}{z} \log M^{+z}$$

where z is the number of electrons exchanged. $E_{M^{+z}}$ is the standard electrode potential. The half cell potential for the Ag/AgCl measuring electrode can also be calculated from the Nernst equation. In this case, the cell potential $E_{Ag/AgCl}$ is equal to its standard electrode potential $E^{\circ}_{Ag/AgCl}$ if the reference electrode is saturated with Cl ions. The cell potential (or the rest potential) developed between the test material and the Ag/AgCl measuring electrode, when both are in contact with the same buffered salt solution, is given by the difference between these half cell potentials, i.e.,

$$E_{cell} = E^{\circ}_{Ag/AgCl} - [E^{\circ}_{M^{+z}} + \frac{.06}{z} \log M^{+z}].$$

It is often desired to relate the change in the rest potential, or corrosion potential, of a solid surface to the adsorption of protein from solution on that surface or to other basic blood-material interactions. The measurement of the rest potential is very simple and was one of the first techniques used to correlate the position of a metal in the electromotive series with its apparent blood reactivity. Some investigators have suggested that metals more negative than +300 mv with respect to the normal hydrogen electrode (wrt NHE) would be thromboresistant when implanted in the blood stream, but others that the rest potential of several metals does not appear to correlate with their blood compatibility.

Rest potentials are easily measured in normal saline or other electrolytes by making the specimens one electrode in a Josephson bridge and with a saturated calomel electrode as the other (reference) electrode as shown in figure 24.

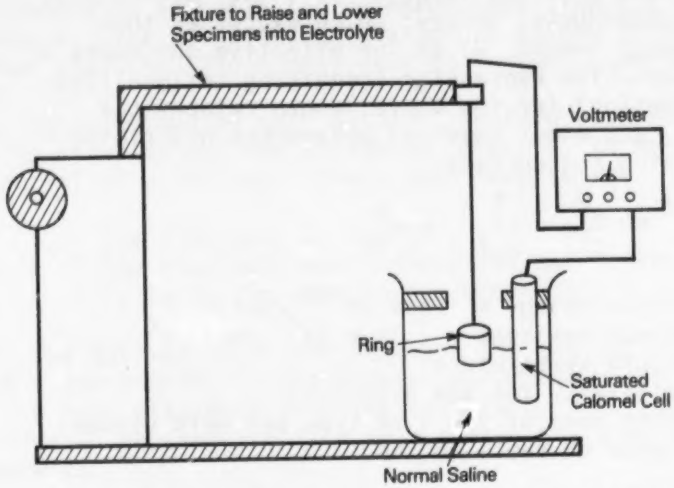


Figure 24. Apparatus for measurement of rest potential.

The rest potential of a material (surface) is best measured using a unity gain high impedance amplifier connected to a digital voltmeter. This electrical arrangement should give an input impedance of 10^{12} Ω for voltage measurements. The unity gain high impedance amplifier thus assures that the electrochemical potential under study is not disturbed electrically, since the measurement current is extremely small.

The rest potentials should be recorded immediately after submersion of the specimen in the saline solution and then again about 10 minutes later. It is interesting to note that the initial rest potential, in most instances, will be different from the value obtained after 10 minutes. In fact, the rest potential of many materials fails to level off to a stable value even after 60 minutes in contact with a conductive solution. No evolution of gas should be noted during any of the measurements.

Zeta Potential (Streaming Potential)

In order to determine the zeta potentials of materials, it is possible to either make electrophoretic mobility measurements on small particles (2 μm diameter) or to flow an electrolyte solution through a capillary made of the same material. The first technique measures the movement of the particles in an electric field and the second measures either the streaming potential or streaming current to determine the zeta potential of the surface. Neither the use of small particles nor capillaries is suitable for the complete list of materials of interest, but rectangular flow cells can be assembled using two flat plates of such materials substituting for the capillary geometry.

In streaming potential experiments, the plane of shear locates the region from which ions are displaced by the flowing electrolyte. The distance of this plane from the layer of potential-determining ions is given by the Debye-Huckel theory, which expresses this distance (called the Debye length, D) as the effective thickness of the diffuse double layer. The expression (requiring rationalized MKS units for its evaluation) for the Debye length relates the term, K^2, where D =K/2, and other physical parameters describing the electrolyte contacting the surface:

$$K^2 = \frac{e^2}{\varepsilon KT} \sum_{i=1}^{n} n_i z^2_i$$

where e is the electron charge = 1.6 x 10^{-19} coulomb
k is the Boltzman constant = 1.38 x 10^{23} joule/K^o
T is the absolute temperature
ε is the permittivity for the solution
n_i is the number ions of the i th type per unit volume
z_i is the valence of the i th ion.

This expression can be rewritten in terms of the ionic strength of the electrolyte, c. During this and evaluating the constant terms in this expression at 21° C gives

$$K = 0.322 \text{ X } 10^{10}\sqrt{c}/\ m^{-1} \ .$$

Converting to angstroms and taking the reciprocal gives the Debye length,

$$D = \frac{3\text{Å}}{\sqrt{c}} \ .$$

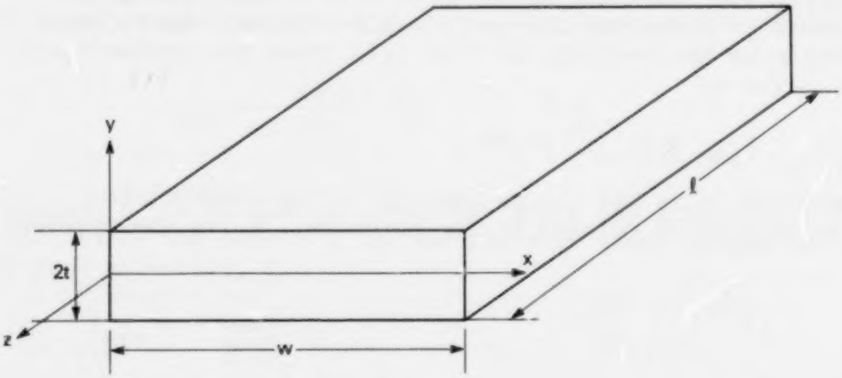
It is usually assumed that the the surface in contact with an electrolyte influences which ions are released from the plane of shear due to electrolyte motion near the surface. If molecular species are present in the electrolyte, then these can adsorb within the potential-determining layer or within the dense part of the double layer and alter the distribution of ions in the diffuse layer such that a new potential at the plane of shear is established. The number of ions in the diffuse layer which may be released when the electrolyte flows past the surface depends on the concentration of the electrolyte. Dilute solutions allow release of greater numbers of ions than do concentrated electrolyte solutions.

The potential at the plane of shear, the zeta potential, can be determined from a measurement of the streaming current or streaming potential which results when an electrolyte flows past the surface. The streaming potential is often orders of magnitude greater than the streaming current at physiologic salt concentrations; hence it is more readily accessible to measurement. Although techniques have been reported which allow the streaming current to be accurately measured, these techniques involve the sinusoidal flow of the electrolyte through a capillary and thus are not generally suitable for research involving the adsorption of proteins. The solutions make multiple passes through the capillary rather than the single pass desired in such experiments.

As described by many authors, certain requirements must be met in order to use the streaming potential to measure the zeta potential of a surface. These requirements are

1. The flow must be laminar, and
2. The flow path must have a surface-to-surface gap which is large compared to the Debye length.

The derivation of the equation for the zeta potential starts with a description of the streaming current which comes about due to the ions displaced from the surface by a moving electrolyte. Consider a rectangular flow cell having the dimensions shown in figure 25.



Sketch of Rectangular Flow Cell (w, Width of Cell; 2t, Thickness of Gap; and ℓ, Length of Cell)

Figure 25. Rectangular flowcell.

The velocity profile between the two parallel flat plates is given by

$$v = \frac{P}{2\eta_o l}(t^2 - y^2)$$

where v is the linear velocity of flow of the liquid in the gap
y is the distance from the center of the gap
p is the pressure drop across the entire length of flow
η is the viscosity of the electrolyte
l is the length of the flow cell
t is half the thickness of the gap

This equation indicates that the velocity profile is parabolic in the y direction of the flow cell gap. The width of the gap, w, is taken to be large compared to the thickness, 2t, so the flow is approximately uniform in the x direction. Ideally, there would be an extra equation to describe the velocity profile in the x direction; however, for simplicity, this will not be considered in this development.

The velocity gradient very close to the flat flow cell walls is given by

$$\frac{dv}{dy}\bigg|_{y=t} = \frac{-Pt}{\eta_o l} \quad .$$

If the Debye length, s, is much less than the thickness of the gap, 2t, then the velocity of the electrolyte at the plane of shear, measured from the solid wall is given by

$$v \doteq \int_o^s \left(\frac{dv}{ds}\right)_{s=o} ds = \frac{Pts}{\eta_o l}$$

where a change of variable has been made with $s = t - y$. The streaming current I_{str} is a product of the charge carried by the moving electrolyte times the velocity of this electrolyte at the plane of shear. Taking the charge in a thin laminar layer, 2w, near the walls of the rectangular flow cell, then the streaming current is given by

$$I_{str} = 2w \int_o^t \rho v \, ds.$$

In this equation, ρ is the charge density in the electrolyte. This charge density is related to the potential, Ψ, in the Poisson equation:

$$\nabla^2 \Psi = - \frac{4\pi\rho}{D} \quad .$$

Substituting the expression for v and Ψ (in one dimension) in the equation for the streaming current gives

$$I_{str} = -2w \int_o^t \frac{D}{4\pi} \frac{d^2\Psi}{ds^2} \frac{Pts}{\eta_o l} \, ds.$$

The assumption is made that D and η_0 are constants equal to their values in the bulk solution. This simplifies the streaming current equation to

$$I_{str} = -\frac{Pwt}{2\pi}\,\frac{D}{\eta_o l}\int_o^t \frac{d^2\Psi}{ds^2}\; s\; ds.$$

The integral can be integrated by parts using the relation

$$\int \xi\, dz = \xi z - \int z\, d\xi$$

with

$$\xi = s \quad \text{and} \quad dz = \frac{d^2\Psi}{ds^2}\, ds.$$

Having done this, the following expressions are derived:

$$\int_o^t \frac{d^2\Psi}{ds^2}\; s\; ds = s\,\frac{d\Psi}{ds}\Big|_o^t - \int_o^t \frac{d\Psi}{ds}\, ds = s\,\frac{d\Psi}{ds}\Big|_o^t - \Psi\Big|_o^t .$$

Considering the potential to be short ranged, it is usually safe to assume that the potential and its gradient are zero at the center of the flow cell. These boundary conditions are

$$\frac{d\Psi}{ds}\Big|_{s=t} = 0 \quad \text{and} \quad \Psi\Big|_{s=t} = 0 .$$

Under these conditions, the expression for the streaming current, rewritten below

$$I_{str} = -\frac{PwtD}{2\pi\eta_o l}\left\{\frac{s\,d\Psi}{ds}\Big|_o^t - \Psi\Big|_o^t\right\},$$

is reduced to the following expression where the potential $\Psi|_{s=o}$ is taken to be the potential at the plane of shear or the well-known zeta potential, ζ.

$$I_{str} = -\frac{PwtD}{2\pi l\eta_o}\,\zeta .$$

This expression for the streaming current indicates that for a negative zeta potential (this does not mean a negative surface potential, because potential determining ions on the surface could alter the potential away from the solid surface), the current in the direction of flow would be positive. That is, it would consist of positive counter ions displaced from the plane of shear by the moving electrolyte. This would make the streaming potential, as discussed below, positive in the direction of fluid flow. The reverse would be true for a positive zeta potential.

This streaming current gives rise to a streaming potential E_{str}. I_{str} is balanced by a back current I_B conducting the charge released from the shear plane back through the electrolyte and the flow cell itself. When there is appreciable conduction through the bulk of the flow cell, because the flow cell is constructed of conductive materials, the equations which are developed must explicitly include this bulk conductivity. Under steady state conditions, the streaming current and this back current exactly balance. Therefore, the following expression is obtained:

$$\frac{PwtD}{2\pi\eta_0 l}\zeta = E_{str}\ \frac{2wt}{l}\ [K_e + \frac{lK_s}{t}] .$$

K_e = Conductivity of electrolyte

K_s = Conductivity of flow cell

Solving for ζ in this equation, we find

$$\zeta = \frac{4\pi\eta_0}{D}\ \frac{E_{str}}{P}\ [K_e + \frac{lK_s}{t}] .$$

In the case where $K_s = 0$, the expression derived is

$$\zeta = 4\pi\ \frac{E_{str}}{P}\ \frac{\eta_0}{D}\ K_e ,$$

which is identical to that given in many texts for a simple circular glass capillary.

Electrodes

The electrodes recommended for most electrochemical characterizations of biomaterials are Ag/AgCl systems encased in an agar salt bridge. The salt bridge is placed in contact with flowing electrolytes, thereby protecting the electrodes from exposure to the solution and also reducing the posibility of their drying out between experiments.

During the preparation of such electrodes, silver wire is inserted through an insulating sleeve and then brushed with steel wool to remove the surface oxide. At the same time, a plate of silver, approximately 1" x 2", is also cleaned with steel wool. This silver plate along with a spare piece of silver wire is then placed in a 0.1 N HCl solution. The silver plate is then made positive and a potential applied between it and the silver wire such that 5 ma flows. After 5 minutes, the silver plate and the silver wire are removed and the silver plate which is now black is once again cleaned with steel wool. The operation just described is to clean the plating solution prior to making the Ag/AgCl electrodes.

With the silver plate and all the silver wires to be made into Ag/AgCl electrodes rinsed free of any steel wool, the silver wires and plate are mounted in the beaker of cleaned electroplating solution. In the case where multiple electrodes are to be used, all silver wires to be plated should be connected together and plated simultaneously by making them positive with respect to the silver plate. A useful plating current is 3 ma, applied for 3 minutes. The Ag/AgCl electrodes so formed are bluish purple in color.

Prior to removing the electrodes from the plating bath, the potential between them should be checked with a high impedance volt meter. Freshly made electrodes typically have a voltage difference of only a few tenths of a millivolt between them. At this point, the electrodes are susceptible to damage if exposed to air. Therefore, all solutions for forming the salt bridge should also be prepared before removing the electrodes for insertion into the electrode holders.

A typical agar salt bridge is made by placing 0.75 gm of purified agar in a beaker containing 25 ml of boiling water. This beaker is within another beaker of boiling water, thereby forming a double boiler arrangement. When the agar is fully dissolved, a measured quantity of KCl is added to form a 0.1 M KCl solution. This mixture is stirred until it is clear. A heated syringe with a fine needle is then be filled with this prepared solution and the solution is injected into the space between one of the Ag/AgCl electrodes which has been inserted into the electrode holder and the inside of the electrode holder. (The Ag/AgCl electrodes should be briefly rinsed in distilled water prior to insertion into the electrode holders.) All electrodes should be prepared in the same way, being careful to place the syringe filled with the prepared agar in hot water to prevent the agar from solidifying.

Electrodes prepared in this way when tested in a saturated KCl solution exhibit very small potential differences between them. (This is typically only tenths of a millivolt.)

Appendix G

Vibrating Electrode System for Measurement of Surface Properties

by S. Aisenberg

Measurement of the surface charge and surface potential of biomaterials will aid in improving the reproducibility of the samples. It will help identify batch variations, and surface contamination, as well as the intrinsic surface potential of various biomaterials. There is no proven relationship between the potential of a surface in air, and the potential of this surface in blood; however, there may be a useful correlation between the surface potential in air and the subsequent observed blood compatibility. Collecting data for various biocompatible materials (and non-compatible materials) may add another factor for comparison with blood compatability.

A device for measurement of surface charge and potential has been built based upon the Kelvin technique of vibrating an electrode perpendicular to the test surface. If the surface potential and the electrode potential are the same, then the electrode is vibrating in a field-free region and no electrical signal is generated. If there is a potential difference then there will be a signal whose phase depends upon the sign of the surface charge and surface potential.

A nulling voltage, V_B, is applied to the test material and adjusted to give an A.C. null on the vibrating electrode. For insulating materials, the back side of the sample is coated with a conducting paint, and then bonded to a conducting substance which is connected to the nulling voltage.

The relationship between the surface charge density, σ (in coulombs m/^{2}), and the nulling voltage, V_B (volts), is given by

$$\sigma = \frac{K \varepsilon_0 V_B}{d}$$

where K is the dielectric constant of the sample

ε_0 is the permittivity of free space
[= 8.854 X 10^{-12} Farad/m (MKS)]

d is the insulator sample thickness (in meters)

As an example, the average charge separation corresponding to a surface potential of -0.1 volts can be calculated for polyurethane.

The dielectric constant reported for various polyurethanes ranges from 5.4 to 7.6; a mean of 6.5 will be used for these calculations. Dielectric constants for polypropylene range from 2.2 to 2.6, while values for polyethlene range from 2.25 to 2.35.

Assume a polyurethane thickness of 4 mils (1.02×10^{-4} meter) and $V_B = 0.1$ volt

then $\sigma(\text{coulomb/m}^2) = (0.1)(6.5)(8.854 \text{ X } 10^{-2})/(1.02 \text{ X} 10^{-4})$

and $\sigma(\text{coulomb/m}^2) = 5.56 \text{ X } 10^{-8}$

or $\sigma(\text{coulomb/cm}^2) = 5.56 \text{ X } 10^{-12}$.

The charge of an electron is 1.602×10^{-19} coulomb; therefore the surface density of electrons is $n = 3.54 \times 10^{7}$ (cm^{-2}).

The average spacing between electrons of the surface charge is $n^{-1/2}$ and is 1.682×10^{-4} cm or 1.682 microns, for the case of 0.1 volt surface potential on 4 mil polyurethane and for the assumptions made.

The operational block diagram of the surface charge measurement system is shown in figure 26. The relative surface potential as seen by the vibrating measuring electrode is processed by a high impedance preamplifier which also serves as a filter turned to the frequency of vibration. This low level signal is further amplified and filtered to produce a sine wave which contains all information needed for synchronous detection.

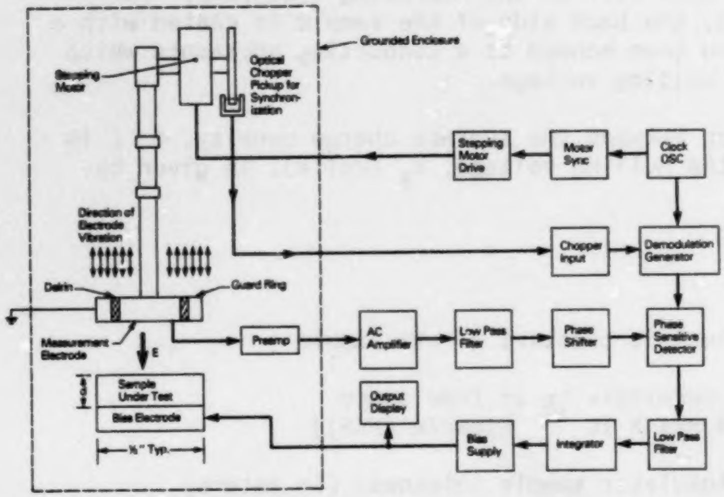

Grounded Enclosure
Stepping Motor
Optical Chopper Pickup for Synchronization
Stepping Motor Drive
Motor Sync
Clock OSC
Direction of Electrode Vibration
Chopper Input
Demodulation Generator
Delrin
Guard Ring
Measurement Electrode
E
Preamp
AC Amplifier
Low Pass Filter
Phase Shifter
Phase Sensitive Detector
d
Sample Under Test
Bias Electrode
Output Display
½" Typ.
Bias Supply
Integrator
Low Pass Filter

Figure 26. Instrumentation for measuring surface charge.

Meanwhile, an optical chopper coupled to the stepping motor generates a phase locked square wave for synchronous demodulation.

After demodulation, the resultant output is further processed and is fed to a bipolar integrator. The integrator, which is driven either positive or negative in response to the vibrating electrode, controls the back bias potential applied to the specimen under test. When the vibrating electrode senses no net surface charge, its output drops to zero causing the integrator to hold at its present level. In this way, the surface potential of the specimen under test is automatically brought to a null state.

This automatic nulling system should be of particular value in determing the state of surface charge on slowly varying surfaces. Resolutions of 10 mV have been achieved, corresponding to a surface charge sensitivity of 10^{-13} coulombs/cm^2 for most polymeric surfaces of about 4 mil thickness.

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